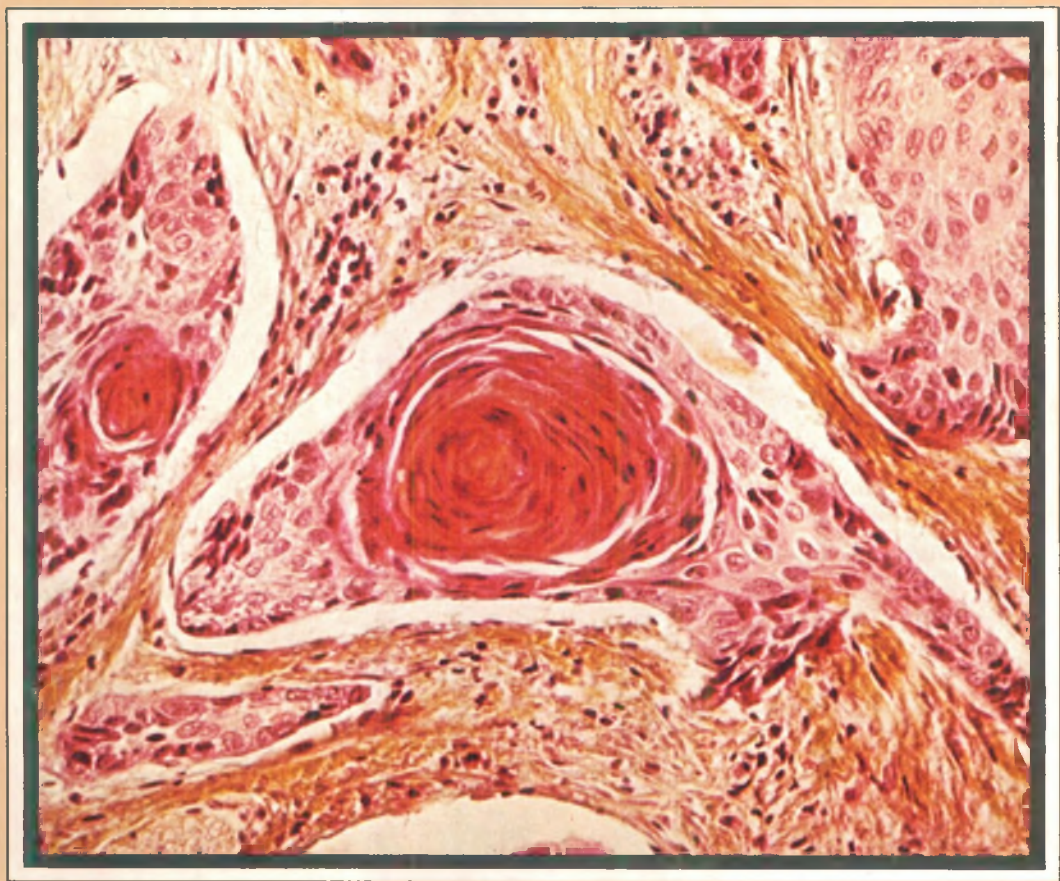


Late Biological Effects of Ionizing Vol. II Radiation

PROCEEDINGS OF A
SYMPOSIUM
VIENNA, 13-17 MARCH 1978



INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1978

The cover picture shows an epidermoid carcinoma, highly differentiated [taken from Histological Typing of Lung Tumours, WHO, Geneva (1967)].

**LATE BIOLOGICAL EFFECTS OF
IONIZING RADIATION
VOL. II**

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THE LATE BIOLOGICAL EFFECTS OF
IONIZING RADIATION
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In two volumes

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FOREWORD

With the rapid rise in the peaceful uses of atomic energy in medicine, industry and power generation, concurrent efforts are being made to protect workers and the general population from exposure to external and internal radiation sources. The mechanisms of the biological effects of ionizing radiation are fairly well elucidated. From the health protection point of view certain hazardous somatic effects which can appear long after the time of exposure are of particular interest. These effects, termed late biological effects, may include possible induction of malignant neoplasms, various types of degenerative diseases, impairment of fertility and cytological abnormalities.

Research and surveys are continuing, under the various national, regional and international programmes, directed towards the gaining of further knowledge of the nature and extent of incidence of these hazardous effects; this work, an integral part of the radiation protection programme, aims to provide the relevant scientific bases for their prevention. Although much work has been done, there are still considerable ambiguities in the qualitative and quantitative aspects as well as in the mechanisms, not to mention the additional complications projected through the inevitable interactions of some chemical and physical agents co-existing in the environment. These factors associated with the late biological effects are of great significance in setting up guidelines for acceptable radiation exposure, and in evaluating the risk/benefit factor for the future promotion of peaceful nuclear applications.

The Symposium on the Late Biological Effects of Ionizing Radiation was convened in Vienna by the International Atomic Energy Agency from 13 to 17 March, 1978. The symposium was attended by about 250 participants from 33 countries and nine international organizations. The principal aim of the symposium was to review the current status of understanding of the late biological effects of ionizing radiation from external and internal sources. Furthermore, the international experts critically evaluated information obtained from epidemiological studies of relevant human population groups, such as atomic bomb survivors, patients receiving medical exposures as well as occupationally exposed radiation workers. Eighty-one papers were presented in 10 sessions which covered epidemiological studies on late effects in human populations exposed to internal and/or external ionizing radiation; quantitative and qualitative data from experimentation on animal models; methodological problems and modern approaches; factors influencing susceptibility or expression of late radiation injury; comparative evaluation of late effects induced by radiation and other environmental pollutants; and problems of risk assessment.

In addition, a discussion was held on the planning of further epidemiological studies of occupationally exposed populations in the light of the projected rapid growth of the nuclear power programme in Member States. The expert group recommended that a national registry system for the dosimetry and medical records of radiation workers be established and co-ordinated internationally in order to facilitate reliable epidemiological surveillance and risk-assessment of the population.

The dose-effect relationship, by which human risk estimates at low doses are derived by extrapolation, was also discussed. Several new models based on theoretical analysis and experimental data were proposed and compared with available human and animal data. The important matter of predicting the combined effects, where the picture is complicated by other environmental factors such as chemical pollutants, was discussed in several papers.

The full text of the papers, together with a record of the discussions, is published in these Proceedings. The symposium brought together international experts with a wide range of interests including the practicalities of radiological protection, health physics, epidemiology, oncology and other relevant medical practices, theoretical and experimental radiobiology, biophysics and dosimetry as well as the regulatory authorities of the national health services. The Proceedings are thus expected to serve as a major source of current information and reference material for the above specialities.

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ANIMAL EXPERIMENTATION

Sessions 2 and 4

LATE BIOLOGICAL EFFECTS OF IONIZING RADIATION AS INFLUENCED BY DOSE, DOSE RATE, AGE AT EXPOSURE AND GENETIC SENSITIVITY TO NEOPLASTIC TRANSFORMATION

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Abstract

LATE BIOLOGICAL EFFECTS OF IONIZING RADIATION AS INFLUENCED BY DOSE, DOSE RATE, AGE AT EXPOSURE AND GENETIC SENSITIVITY TO NEOPLASTIC TRANSFORMATION.

A most comprehensive investigation is in progress at the Los Alamos Scientific Laboratory to study the late biological effects of whole-body exposure to gamma irradiation as they may be influenced by total dose, dose rate, age at exposure and genetic background. Strain C57B1/6J mice of four age groups (newborn, 2, 6, and 15 months) were given five doses (20, 60, 180, 540, and 1620 rads) of gamma rays, with each dose being delivered at six dose rates (0.7, 2.1, 6.3, 18.9, 56.7 rads/day and 25 rads/min). Forty to sixty mice were used in each of the approximately 110 dose/dose-rate and age combinations. The study was done in two replications with an equal number of mice per replication. Strain RF/J mice were used in a companion study to investigate the influence of genetic background on the type and magnitude of effect. Results of the first and second replications of the 15-month-old age group and data on the influence of genetic background on biological response have been completed, and the results show no significant life shortening within the dose and dose-rate range used. It was also concluded that radiation-induced neoplastic transformation was significantly greater in mice with a known genetic sensitivity to neoplastic disease than in mammals which do not normally have a significant incidence of tumours.

1. INTRODUCTION

Late biological effects of whole-body exposure to ionizing radiations from external sources, expressed in terms of life shortening, have been studied extensively for many years. Variables such as total dose received, dose rate used to administer the exposure dose, age of the animal at time of exposure, and genetic background of the animals have been studied separately and, to some degree, with two variables

TABLE II. AGE GROUPS USED IN DOSE/DOSE-RATE COMBINATIONS FOR LATE BIOLOGICAL EFFECTS OF RADIATION EXPOSURE STUDIES

Dose (rads)	Dose rate (rads per 24-hour day)					Rads per minute 25
	0.7	2.1	6.3	18.9	56.7	
20			NB ^a		NB	NB
	2	2	2	2	2	2
	6	6	6	6	6	6
	15	15	15	15	15	15
60	NB		NB		NB	NB
	2	2	2	2	2	2
	6	6	6	6	6	6
	15	15	15	15	15	15
180	NB		NB		NB	NB
	2	2	2	2	2	2
	6	6	6	6	6	6
	15	15	15	15	15	15
540	NB		NB		NB	
	2	2	2	2	2	
		6	6	6	6	
			15	15	15	
1620			NB			
			2	2	2	
			6	6	6	
				15	15	
Control	NB					
	2					
	6					
	15					

^a Newborn.

TABLE III. MANN-WHITNEY TEST OF THE CONTROL AND TREATED GROUPS OF REPLICATIONS 1 AND 2 TO DETERMINE THE VALIDITY OF COMBINING DATA FROM THE TWO REPLICATIONS

Dose (rads)	Dose rate		P-value
	(R/day)	(R/min)	
0	0.0		0.077
20	0.7		0.670
60	0.7		0.160
	0.7 ^a		
20	2.1		0.430
60	2.1		0.850
180	2.1		0.760
20	6.3		0.870
60	6.3		0.960
180	6.3		0.032
540	6.3		0.500
20	18.9		0.630
60	18.9		0.005 ^b
180	18.9		0.190
540	18.9		0.610
20	56.7		0.500
60	56.7		0.017 ^b
180	56.7		0.540
540	56.7		0.760
1620	56.7		0.510
20		25	0.110
60		25	0.0001 ^b
180		25	0.200
540		25	0.047

^a Only one replication.

^b Significant difference between replications at the 0.05 level.

Mice are somewhat unique in this regard and are different from the human female. Sterility of the female mammal resulting from an irreparable disturbance of the normal ovarian function results in an imbalance and/or diminished levels of ovarian hormones. The primary and/or secondary effects of this permanent hormonal imbalance on the incidence of killing diseases are not known. For this reason, life reduction from radiation exposure in the female mouse may not be representative in either degree or kind to that which might be expected in other mammalian species, including the human primate.

TABLE IV. MEAN AND MEDIAN LIFE-SPAN VALUES OF CONTROL AND TREATED MICE IN LATE BIOLOGICAL EFFECTS OF RADIATION EXPOSURE STUDIES

Dose (rads)	Dose rate (rads per 24-hour day)					Rads per minute
	0.7	2.1	6.3	18.9	56.7	25
20	929 ± 147 954	895 ± 138 920	939 ± 135 936	919 ± 139 925	930 ± 163 928	848 ± 124 830
60	894 ± 127 905	923 ± 126 935	876 ± 139 895	891 ± 114 910	861 ± 108 870	838 ± 134 774
180	881 ± 92 884	888 ± 148 923	859 ± 174 867	897 ± 124 938	863 ± 109 835	840 ± 144 844
540			866 ± 150 876	921 ± 111 930	899 ± 139 922	852 ± 148 829
1620				847 ± 134 882	872 ± 124 885	
Control	870 ± 139 ^a 893					

^a Mean life span for strain C57B1/6J mice at their source (Jackson Laboratory, Bar Harbor, Me) is reported to be 670.

Two exposure facilities with cobalt-60 point sources were used to administer the dose/dose-rate exposure combinations required. All animals were checked twice a day for lethality. Dead and moribund mice were examined post-mortem, and tissues were saved from all abnormal conditions for histopathology studies.

This investigation is currently in progress, and data from replications 1 and 2 of only the 15-month age group are available at this time.

3. RESULTS

A preliminary analysis of these data has been completed and is presented here. Data from replications 1 and 2 were analysed separately for significant differences in life span. Three of the radiation-treated groups in replication 1 showed life spans significantly different (at the 5% level of significance) from the control group. The treated groups receiving 20 rads at either 0.7 R/day or 6.3 R/day, and 60 rads at 0.7 R/day, lived significantly longer than the controls.

In replication 2, one treated group (180 rads at 25 R/min) showed a mean life span significantly different and, in this case, shorter than that of control mice.

TABLE V. MANN-WHITNEY TEST OF DIFFERENCES BETWEEN COMBINED CONTROL VERSUS TREATED GROUPS OF THE TWO EXPERIMENTAL REPLICATIONS

Dose (rads)	Dose rate		P-value
	(R/day)	(R/min)	
20	0.7		0.014 ^a
60	0.7		0.380
180	0.7		0.930
20	2.1		0.290
60	2.1		0.030
180	2.1		0.350
20	6.3		0.020 ^a
60	6.3		0.840
180	6.3		0.750
540	6.3		0.870
20	18.9		0.090
60	18.9		0.380
180	18.9		0.240
540	18.9		0.100
1620	18.9		0.370
20	56.7		0.090
60	56.7		0.450
180	56.7		0.650
540	56.7		0.440
1620	56.7		0.840
20		25	0.400
60		25	0.220
180		25	0.130
540		25	0.560

^a Significant at the 0.05 level (< 0.025 significant difference at the 5% level).

The two replications were compared to see if they could be combined. Table III gives, for each treatment, a P-value (i.e. the level at which the test would be barely significant) from the Mann-Whitney test. Three out of the 24 groups of the two replications differed significantly from each other. There was nothing systematic in the three differences observed and, since 5% of the differences are expected to be significant, it was concluded that no real differences existed which would preclude combining the data of the two replications.

The data from the two replications were pooled, and the mean and median life-span values are shown in Table IV. The Mann-Whitney test was used to check

TABLE VI. TUMOUR INCIDENCE (%) OF CONTROL AND TREATED MICE IN LATE BIOLOGICAL EFFECTS OF RADIATION EXPOSURE STUDIES (TOTAL/MALIGNANT)

Dose (rads)	Dose rate (rads per 24-hour day)					Rads per minute 25
	0.7	2.1	6.3	18.9	56.7	
20	0/0	2/2	0/0	0/0	0/0	8/0
60	4/0	0/0	0/0	0/0	0/0	3/3
180	0/0	0/0	2/2	3/0	8/5	5/3
540			5/0	6/6	6/6	6/3
1620				3/0	6/6	
Control	10/7					

for differences between treatments and controls for all the combined data (i.e. replications 1 and 2 were combined for the control group, and replications 1 and 2 were combined for each of the treatment groups). Table V gives a P-value for each test of treatment versus control. Small P-values (i.e. say, < 0.05) indicate significant differences. The mean life-span value of the control group (870 ± 139) was 200 days longer than reported by the Jackson Laboratory where these mice were obtained. Two significant differences were found among the 24 comparisons (20 rads at 0.7 R/day and 20 rads at 6.3 R/day) and, in each case, it was the treated group which had longer life spans than the control group.

Gross and histopathology observations made on these animals at death showed the cause of death, in most cases, to be nonspecific. The incidence of tumours in each group is shown in Table VI. Both the total number and malignant tumours were greatest in the control group. There did not appear to be any relationship between tumour incidence and dose, dose rate or life span among the 24 groups observed.

4. DISCUSSION

In earlier studies, female mice of two strains with different malignant tumour frequencies at death were observed for life shortening and cause of death after exposure to X-ray doses from 200 to 1200 rads.¹ Strain RF/J mice (with 43%

¹ These doses were given in 200-rad fractions at 7-day intervals and at a dose rate of 200 R/min.

leukaemia at death in controls) showed dose-related life shortening, accompanied by an increase in frequency of neoplastic disease with increased doses of whole-body X-ray exposure. Strain C57B1/6J mice (with nonspecific pathology at death in controls) showed less life shortening, with no increase in frequency of neoplastic disease in X-irradiated animals.

We conclude that, within the range of this experiment, whole-body gamma-ray exposure does not affect life expectancy adversely. Further, a mammalian species and strain which is not prone to neoplastic disease under normal conditions may be expected to be significantly more resistant to radiation-induced neoplastic transformation than one which has exhibited a genetic sensitivity to neoplastic disease.

These negative results may be attributed to the age, sex or strain of mice used in this investigation or to a combination of these characteristics. The influence of age will be determined when data from the three younger age groups become available.

ACKNOWLEDGEMENT

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DISCUSSION

R.L. ULLRICH (Chairman): With regard to the lack of life shortening in your C57B1/6J mice after irradiation at age 15 months, would you not see similar results even with a more radiation-sensitive strain such as RF/J?

J.F. SPALDING: No, I don't think so. In current and earlier studies with RF/J mice, with a normally high tumour incidence at death, I have observed life shortening which is related to dose. However, there has also been an increase in the incidence of tumours and/or earlier occurrence of these tumours in this tumour-sensitive strain. I should point out, however, that the C57B1/6J mice that I have reported on were 15 months of age when exposed, whereas the RF/J mice were 3–4 months old when exposed. Complete and convincing evidence on the role that genetic sensitivity to neoplastic disease may play in radiation-induced life shortening will be available when the data from studies on our three younger age groups are complete.

M. DELPLA: Many of the groups shown in Table VI do not contain a single benign tumour, and malignant tumours are even rarer. Once again I ask myself whether irradiation may not have exerted a protective antitumoral effect. Have you observed any statistically significant differences?

J.F. SPALDING: Two irradiated groups (20 rads at 0.7 R/d and 20 rads at 6.3 R/d) had significantly longer life spans than controls, but we have not examined tumour incidence for significant differences. The control group did, however, have more tumours than any one of the treated groups.

R.E. LINNEMANN: To what do you attribute the fact that the control groups had shorter life spans than some of your irradiated groups?

J.F. SPALDING: I have no answer to this question. It is common knowledge that very low doses of ionizing radiation have extended life span in many experiments performed over the past quarter of a century. Perhaps at this age (past middle age) small doses of ionizing radiations do indeed act as a stimulant to the vital life-sustaining tissues.

G. COWPER: In view of the apparent absence of adverse radiation effect in the low-dose groups when compared with a single control group, would it not be prudent to include several control groups in such studies?

J.F. SPALDING: For all practical purposes we did have two control groups, one in each of two replications. The mean life spans of these two control groups did not differ from each other.

MAMMARY CARCINOGENESIS IN RATS AFTER X- AND NEUTRON IRRADIATION AND HORMONE ADMINISTRATION

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Abstract

MAMMARY CARCINOGENESIS IN RATS AFTER X- AND NEUTRON IRRADIATION AND HORMONE ADMINISTRATION.

The relatively high estimates of the incidence per unit dose of radiation-induced breast cancer in women are derived from a few studies on groups of women who were exposed to large doses of radiation. In view of the implications of these observations for the risk estimates of low dose exposures, such as those employed in mammography, it is of great importance to obtain quantitative information on the dose-effect relationships for mammary gland carcinogenesis in experimental models. Three rat strains, Sprague-Dawley (SD), Wistar WAG/Rij and Brown Norway (BN), with expected different susceptibilities for the induction of mammary cancer, have been irradiated with 300 kV X-rays and mono-energetic neutrons with energies of 0.5, 4 and 15 MeV. For each type of radiation and different dose levels four subgroups have been included in the study, intact females, intact females treated with oestrogen, hysterectomy ovariectomized females and hysterectomy ovariectomized females treated with oestrogen. Increased hormone levels were obtained by subcutaneous implantation of pellets with oestradiol-17 β (E₂). A total of 5000 animals are included in the study. In the irradiated groups the first tumours generally appeared between 10 and 12 months after irradiation. For the non-irradiated control groups the latent period is considerably longer (e.g. 21.5 months in the Sprague-Dawley). The cumulative mammary tumour induction is presented as a function of radiation quality, absorbed dose and time after exposure. The dose effect curves derived from these data are used to determine the relative biological effectiveness (RBE) for the different neutron beams. The preliminary results of this study indicate that there are considerable differences in susceptibility for tumour induction by ionizing radiation in the three rat strains.

1. INTRODUCTION

Studies on radiation carcinogenesis are important for assessing risks of ionizing radiation during occupational and accidental exposure. Furthermore, this type of research is of interest for a risk-benefit analysis of diagnostic procedures involving

small doses of ionizing radiation. In this connection mammography is at present under intensive discussion [1]. The human data available for X- and gamma radiation all pertain to large doses and show relatively large variations [2] so that it is difficult to extrapolate to the range of low doses, as required for risk estimates of X-ray diagnosis and industrial radiation exposure. Since oestrogenic hormones have been reported to promote radiation-induced carcinogenesis, and because a large proportion of women at risk is on contraceptive oestrogen medication, it is also important to investigate the combined effects of radiation and oestrogens.

Since 1973 a programme on carcinogenesis of the rat mammary gland has been carried out at TNO with the following three aims: (a) investigation of the nature of the dose-effect relationships for X-rays and neutrons, (b) study of the synergistic interaction of oestrogen administration and neutron irradiation, and (c) determination of the RBE of different neutron beams to assess the relative risk of neutron irradiation. Three rat strains, Sprague-Dawley (SD), Wistar WAG/Rij and Brown Norway (BN) with expected different susceptibilities for the induction of mammary cancer have been irradiated with X-rays and mono-energetic neutrons with energies of 0.5, 4 and 15 MeV.

For the artificial hormone, diethylstilbestrol (DES), a synergistic interaction between hormone administration and X-irradiation has been reported [3, 4]. In our studies on the effects of the combined administration of oestrogens and radiation, the natural hormone oestradiol-17 β (E_2) has been introduced to ensure similarity with the current situation in women on contraceptive oestrogen pills. In the present contribution the preliminary results of the programme are discussed. The dose-effect relations derived for the different radiation qualities have been employed to determine the relative biological effectiveness (RBE) for the different neutron beams.

2. MATERIALS AND METHODS

The three rat strains are bred in closed colony systems at Rijswijk. The WAG/Rij rats were obtained from the Glaxo Laboratory, England, in 1953 (40th generation at the time of the experiment), the BN/Bi rats from Microbiological Associates, Bethesda, Maryland, United States of America, in 1963 (23rd generation) and Sprague-Dawley from the University of Ulm in 1973 (6th generation).

Hormone administration was performed by subcutaneous implantation of E_2 cholesterol pellets into the dorsal region of the neck. In the initial experiments the E_2 was administered at the same concentration as applied for DES; however, at these E_2 levels considerable toxicity was observed. The physical condition of the animals deteriorated and a large number of mammary and pituitary tumours

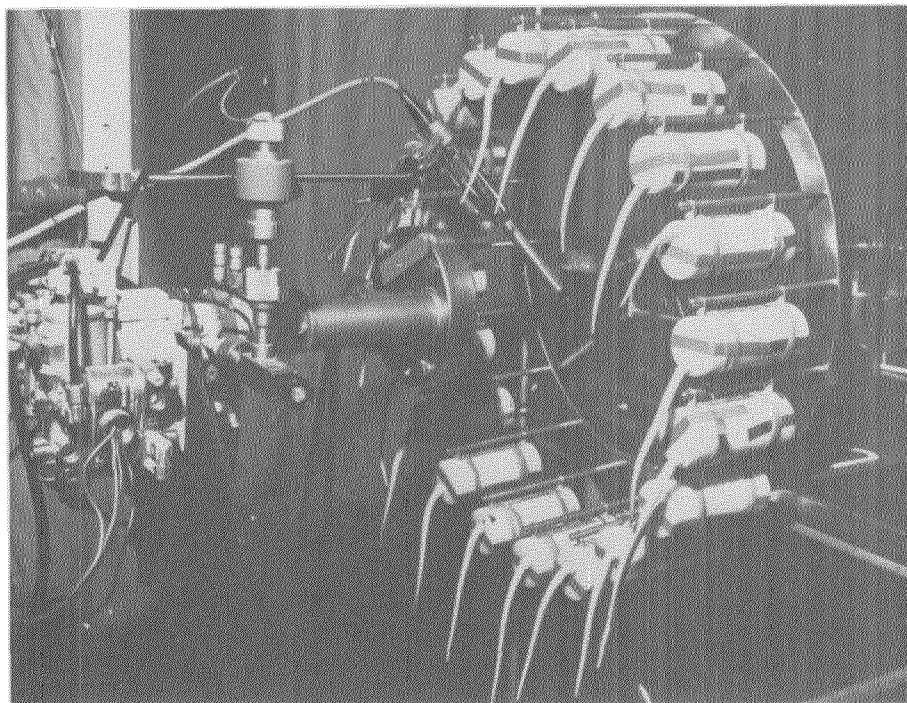


FIG.1. Exposure arrangement for rat irradiations with 4 MeV and 0.5 MeV neutrons, produced by the $d+D$ and $p+T$ reaction respectively.

was observed after latent periods of 10 to 12 months. The amount of oestrogen to be administered was therefore decreased to 2 mg, and the method of pellet production was slightly modified. Fused E_2 pellets in a paraffin-cholesterol base provide a more constant release of the hormone than E_2 pellets pressed with cholesterol as the single carrier. Information on the toxicity experiments and the monitoring of the hormone levels in the blood plasma can be found elsewhere [5].

Neutron beams with energies of 0.5, 4 and 15 MeV were produced with a double-belt Van de Graaff K2N-3750 positive ion accelerator through the $p+T$, $d+D$ and $d+T$ reactions respectively. Special exposure arrangements were constructed to allow bilateral or multilateral irradiations with the aim of achieving uniform dose distributions over the mammary gland tissue. In view of the dependence of dose rates and neutron energy on the angle of emission for $d+D$ and $p+T$ neutrons, it was decided to irradiate the animals on a fixture rotated coaxially with the ion beam axis. This procedure averages the variations in fluence rate around rings of constant angle which are due to aberrations produced by non-symmetrical target structures. The irradiation arrangement shown in Fig.1 has

TABLE I. DISTRIBUTION OF EXPERIMENTAL GROUPS OF THREE DIFFERENT RAT STRAINS FOR THE MAMMARY CARCINOGENESIS PROGRAMME

Exposure condition	WAG/Rij and Brown Norway (BN)	Sprague Dawley (SD)
	For each type of radiation and different dose levels, four subgroups were introduced (a) Intact females (b) Intact females with oestrogen (c) Hystero-ovariectomized females (d) Hystero-ovariectomized females with oestrogen	
Non-irradiated controls	—	—
X-rays	25, 100 and 400 rads	10, 30, 100 and 200 rads
0.5 MeV neutrons	5, 20 and 80 rads	2, 8 and 32 rads
4 MeV neutrons	10, 30 and 100 rads	4, 12 and 40 rads
15 MeV neutrons	15, 50 and 150 rads	5, 15 and 50 rads

the additional advantage of providing multilateral irradiation, since the animal cages remain in the same vertical position during the rotation of the fixture.

The average absorbed dose at the site of the mammary glands, which are considered to lie within the first 8 mm from the body surface, has been determined with tissue equivalent ionization chambers and GM counters [6]. For the irradiations with p+T, d+D and d+T neutrons the average gamma-ray contribution relative to the total absorbed dose was determined to be equal to 0.04, 0.15 and 0.10 respectively. Allowance has been made for the contribution from gamma rays; for the neutron irradiations the dose values refer to the neutron doses only.

The rats were irradiated at 8 weeks of age with single doses of either X-rays or mono-energetic fast neutrons. The hystero-ovariectomies were performed at 4 weeks of age, and implantation of oestradiol pellets was done at 6–7 weeks of age. All animals were inspected weekly and the presence of mammary tumours was carefully noted as to time of appearance, size and location. The animals are allowed to live out their natural lifespan and are killed when moribund. Severely autolysed or partially cannibalized rats were discarded. A complete necropsy is performed and representative sections from all tissues are examined histologically. Special attention is paid to the numbers and histological types of mammary tumours as well as to the presence of non-neoplastic mammary gland lesions.

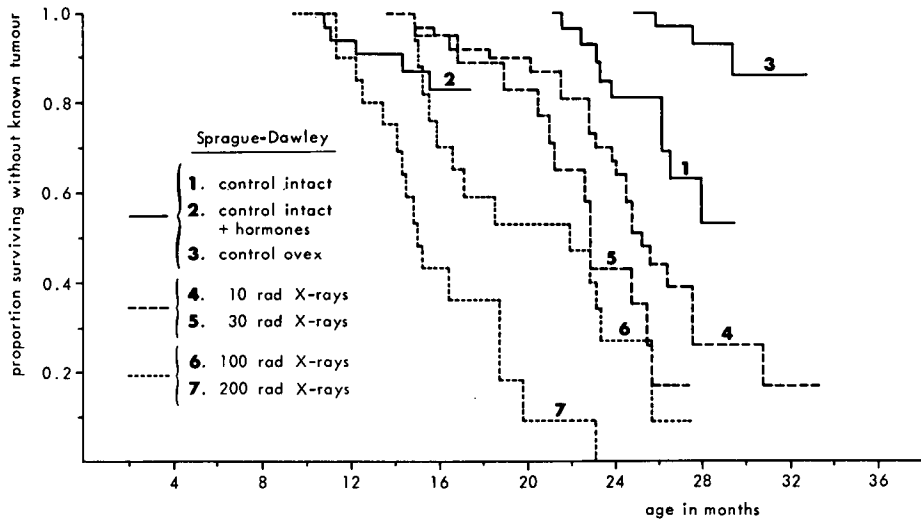


FIG.2. Proportion of Sprague-Dawley rats surviving after X-irradiation without mammary tumours as a function of time.

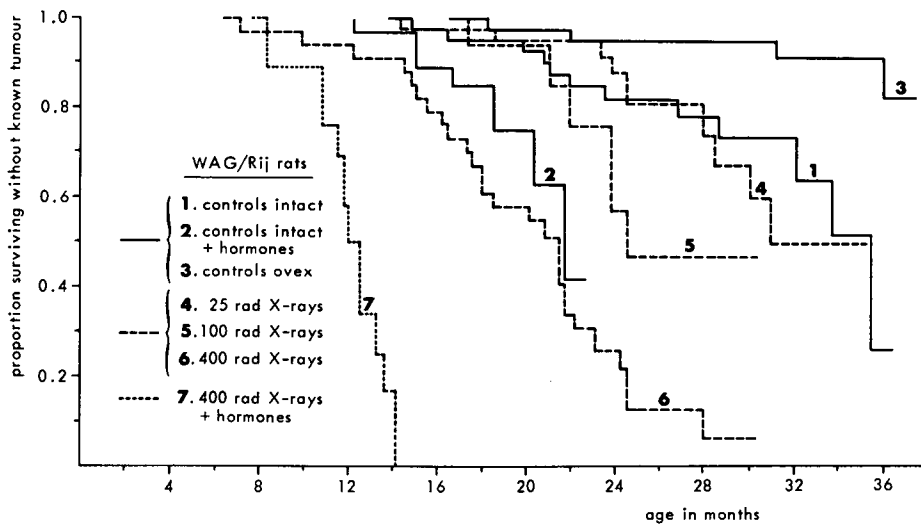


FIG.3. Proportion of WAG/Rij rats surviving after X-irradiation without mammary tumours as a function of time.

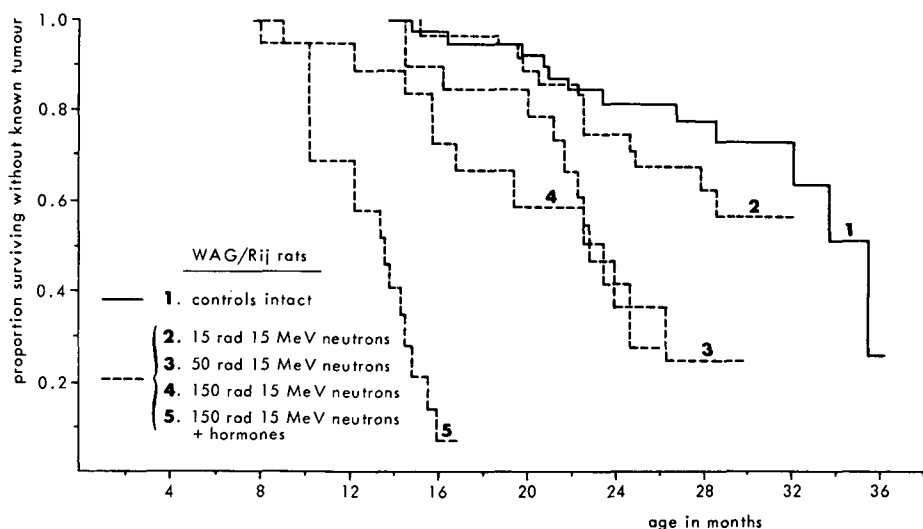


FIG.4. Proportion of WAG/Rij rats surviving after 15 MeV neutron irradiation without mammary tumours as a function of time.

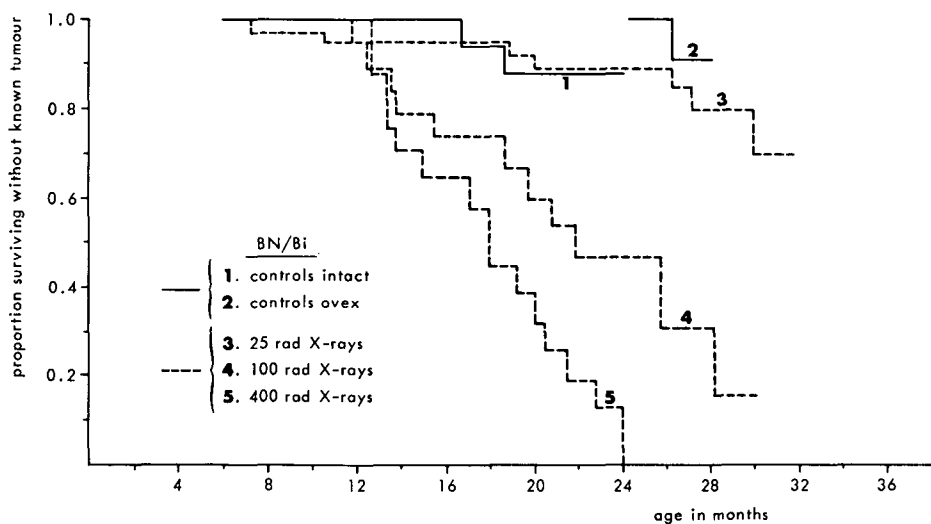


FIG.5. Proportion of Brown Norway rats surviving after X-irradiation without mammary tumours as a function of time.

3. RESULTS

A total number of 5000 animals has been introduced into the study; the distribution of the animals over the different experimental groups is given in Table I. In the irradiated, non-hormone treated groups the first tumours have appeared after latent periods of 10 to 12 months. In some cases soft nodules were palpated in the mammary gland region; some of these nodules disappeared in the course of time. Biopsies were taken from such nodules and histological examination showed that they consisted of dilated ducts filled with secretory material. This finding allowed us to disregard such nodules in the scoring of tumour incidence.

All results are stored in a Data General, Eclipse C/330 computer. The histological examinations of approximately 2500 animals have been completed, and for this group the proportion of rats surviving without known tumour has been calculated according to a life table analysis [7, 8]. The proportion of animals surviving past time t_k without known tumour is recursively calculated from:

$$P(t_k) = P(t_{k-1}) \cdot \frac{n_k - \delta_k}{n_k}$$

For this calculation distinct observation times t_k are considered, $P(t_0) = 1$, n_k is the number of animals at risk during the interval (t_{k-1}, t_k) , being the number of animals alive without diagnosed tumour at t_{k-1} and δ_k is the number of animals showing a first tumour in the interval (t_{k-1}, t_k) . In a preliminary evaluation [9] of the first results on mammary carcinogenesis the cumulative tumour prevalence has been calculated as $1 - P(t_k)$.

A few examples of the life table curves are presented in Figs 2–5 for the three different rat strains for the controls and after irradiation with X-rays and 15 MeV neutrons. The following preliminary conclusions can be drawn from the life table curves:

- (a) The cumulative prevalences for spontaneous tumour induction in the control groups of SD, WAG/Rij and BN rats at an age of 30 months amount to 47, 26 and 12% respectively.
- (b) Initially the WAG/Rij strain was expected to be of intermediate susceptibility for mammary carcinogenesis; however, in accordance with results from other studies [10] our current data suggest that an increased incidence is appearing in the older age groups for this strain.
- (c) The latent periods are longer than described previously by other investigators [4, 11]. In the Sprague-Dawley rats the first mammary tumours in the non-irradiated control groups are observed at an age of 21.5 months.

TABLE II. PERCENTAGE INCIDENCE OF MAMMARY TUMOURS IN FEMALE RATS

	Sprague-Dawley (SD)		WAG/Rij		BN/Bi	
	Intact	Ovex ^a	Intact	Ovex	Intact	Ovex
Control non-irradiated	29 (9/31) ^b	10 (3/30)	27 (10/37)	0 (0/31)	11 (2/19)	2 (1/51)
<i>X-rays</i> (rads)						
25 (SD 10)	69 (20/29)	4 (1/25)	28 (10/36)	0 (0/20)	16 (4/25)	0 (0/30)
100 (SD 30)	71 (12/17)	7 (1/15)	35 (6/17)	11 (1/ 9)	56 (9/16)	5 (1/22)
400 (SD 100)	75 (12/16)	8 (1/12)	76 (25/33)	5 (1/19)	87 (14/16)	9 (1/11)
(SD 200)	86 (12/14)	6 (1/16)	— —	— —	— —	— —
<i>Neutrons</i> , 0.5 MeV (rads)						
5 (SD 2)	12 (3/24)	0 (0/29)	15 (4/27)	9 (2/22)	13 (4/30)	0 (0/35)
20 (SD 8)	50 (7/14)	0 (0/12)	31 (4/13)	0 (0/ 3)	19 (3/16)	0 (0/19)
80 (SD 32)	59 (10/17)	13 (2/15)	56 (9/16)	0 (0/11)	41 (7/17)	6 (1/17)
<i>Neutrons</i> , 15 MeV (rads)						
15 (SD 5)	40 (12/30)	10 (3/31)	36 (13/36)	12 (3/25)	24 (7/29)	8 (2/25)
50 (SD 15)	65 (11/17)	0 (0/10)	58 (11/19)	21 (3/14)	53 (8/15)	0 (0/18)
150 (SD 50)	90 (18/20)	27 (4/15)	56 (10/18)	6 (1/18)	78 (14/18)	0 (0/19)

^a Ovex = hysterio-ovariectomized^b The number of rats with mammary tumours/total number of rats per group examined are given in parentheses.

- (d) For the hysterio-ovariectomized animals the tumour prevalence is considerably lower than for the intact control group.
- (e) In the hormone-treated, non-irradiated animals the tumours occur earlier than in the normal controls. The hormone-treated animals are still under observation; at this moment no conclusion is available about the final values of cumulative prevalence for this group.
- (f) Also in the hormone-treated irradiated animals the tumours appear earlier than in the parallel groups of non-hormone treated animals. Information about the multiplicity of hormone-induced tumours is not yet available.

The number of rats bearing mammary tumours observed up to the present are listed in Table II, as a percentage of the total number of rats examined in each group. As can be seen from the table, the incidence of spontaneously occurring mammary tumours in the non-irradiated control rats was approximately equal in Sprague-Dawley (29%) and WAG/Rij rats (27%), and was 11% in the BN/Bi strain. The increasing tumour incidence with increasing doses is clearly evident for the three strains and with both types of irradiation. On the basis of total tumour incidence over the observation period the WAG/Rij strain can be seen to take, in most situations, an intermediate position between the highly susceptible Sprague-Dawley strain and the least susceptible BN/Bi strain. The dramatic sparing effect of early ovariectomy on the occurrence of mammary tumours in the three strains is evident from the table.

Most mammary tumours observed in rats were histologically classified as benign: 83% for Sprague-Dawley, 57% for WAG/Rij and 88% for BN/Bi. Detailed description of the various histological types of tumours will be published elsewhere. By far the most frequently encountered type was the fibroadenoma. Adenomas, either of a lobular or intraductal papillary type, and fibromas were far less common. The most common types of carcinomas were simple adenocarcinomas and papillary adenocarcinomas or mixtures of the two. Cribriform and anaplastic carcinomas were rare. Spindle cell sarcomas of the mammary gland were extremely rare, as they were found in only 14 animals of the entire series examined.

Although the percentage of rats with mammary tumours increased following irradiation, the ratio of rats with malignant tumours to the total number of tumour-bearing rats did not increase when compared with the controls. Thus, irradiation, regardless of the type, increased the number of both benign and malignant mammary tumours. With respect to the occurrence of multiple mammary tumours in individual rats, it can be stated in general terms that, with increasing radiation dose, regardless of type, a slightly but definitely increased tendency to multiple tumour development was seen in most groups (see Table III).

TABLE III. OCCURRENCE OF MULTIPLE MAMMARY TUMOURS IN INTACT FEMALE RATS

	Sprague-Dawley (SD)			WAG/Rij			BN/Bi		
	Number with n tumours			Number with n tumours			Number with n tumours		
	n = 1	n = 2	n = 3	n = 1	n = 2	n = 3	n = 1	n = 2	n = 3
Control	7	2	0	10	0	0	2	0	0
<i>X-rays</i> (rads)									
25 (SD 10)	13	7	0	9	1	0	4	0	0
100 (SD 30)	8	2	2	4	2	0	9	0	0
400 (SD 100)	8	2	2	13	7	5	11	3	0
(SD 200)	7	4	1	—	—	—	—	—	—
<i>Neutrons</i> , 0.5 MeV (rads)									
5 (SD 2)	3	0	0	3	0	1	4	0	0
20 (SD 8)	6	1	0	3	1	0	2	0	1
80 (SD 32)	7	2	1	5	2	2	5	2	0
<i>Neutrons</i> , 15 MeV (rads)									
15 (SD 5)	8	3	1	12	1	0	7	0	0
50 (SD 15)	9	1	1	9	2	0	7	1	0
150 (SD 50)	13	4	1	7	2	1	9	4	1

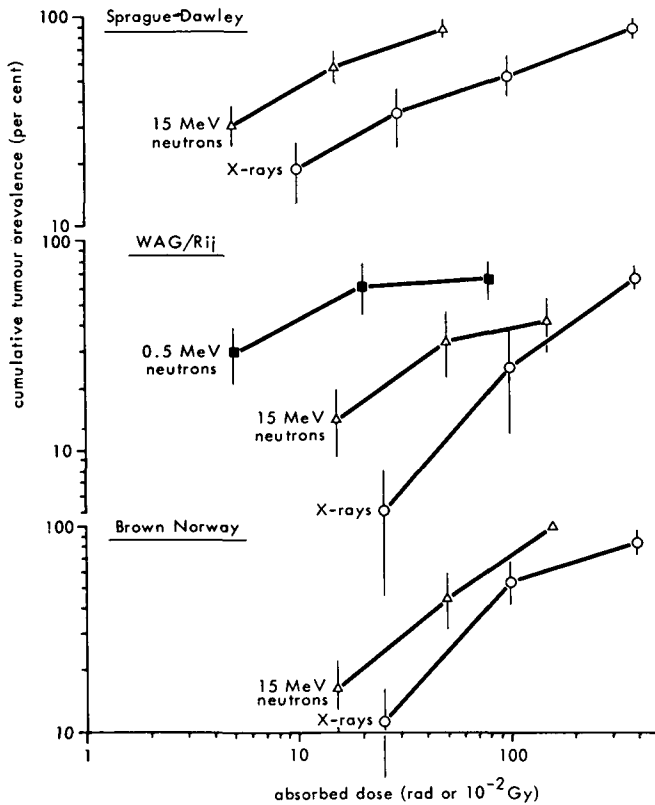


FIG. 6. Cumulative mammary tumour prevalence as a function of absorbed dose for three rat strains after irradiation with X-rays, 0.5 MeV neutrons and 15 MeV neutrons.

TABLE IV. DOSES FOR 30% TUMOUR PREVALENCE (EXAMINED AT 22 MONTHS OF AGE) AND RBE VALUES FOR MAMMARY CARCINOGENESIS AFTER 15 MeV NEUTRON IRRADIATION

Rat strain	Absorbed dose (rad or 10^{-2} Gy) for 30% prevalence		RBE
	X-rays	Neutrons	
Sprague-Dawley	22	4.8	4.6
BN/Bi	60	32	1.9
WAG/Rij	130	44	3.0

4. DISCUSSION

In order to derive dose-effect relationships from the life table curves, the cumulative tumour prevalence has to be scored at a time when the number of spontaneously occurring tumours is still very low. In Fig.6 the cumulative mammary tumour prevalence determined at an age of 22 months for the three rat strains is given as a function of absorbed dose on a log-log plot. The standard errors in the individual prevalence results are calculated with the Kaplan and Meier formalism [7] based on a binomial distribution. The shape of the dose-effect relationship for mammary carcinogenesis and the biophysical interpretations are at present under discussion [12–14]. Before the fundamental implications of our data can be evaluated the information on the number of neoplasms per animal in relation to the dose has to be completed. From the dose-effect curves in Fig.6, the absorbed doses that are required to produce 30% prevalence can be derived. These doses are given in Table IV, together with the RBE values obtained for this level of prevalence. When the scoring of the cumulative tumour prevalence is performed at 22 months of age, and the 30% level is taken as the biological end-point, the Brown Norway rats show an intermediate susceptibility for mammary carcinogenesis after X-irradiation and after 15 MeV neutron irradiation. It can be concluded that the time course of tumour appearance can vary considerably for various rat strains. Consequently the criterion for the degree of susceptibility has to be handled with care. Further statistical analysis has to be performed before we can conclude that the RBE values for the three strains are significantly different. For 0.5 MeV neutrons an RBE value of approximately 20 can be calculated for the WAG/Rij rats at a cumulative prevalence level of 30%.

The present report reflects the current status of our programme on carcinogenesis of the rat mammary gland. In the near future analysis of tumour prevalence will also be performed for parallel groups which were hysterectomy and/or oestrogen treated. Special emphasis will be put on the determination of latency periods, single or multiple occurrence of tumours, histological classification of tumours and dose-effect relationships. Recently, additional experiments have been initiated with fractionated X- and neutron irradiation of rats at one week and one month intervals in order to obtain quantitative information on the risk of repeated low-level exposure to ionizing radiation of different radiation quality.

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DISCUSSION

H.H. VOGEL: You have shown us RBE figures ($D_{X\text{-rays}}/D_{\text{other radiation}}$ for the same effect) for three strains of rats and to several energies of neutrons. You have published results previously for doses at the 20% tumour level. Today you have given us similar RBE figures found at the 30% level. These are high neutron doses. For man it is important to indicate RBE figures following low neutron doses. Would you care to comment on this problem? Do you have any data yet that would allow you to predict RBE values at low neutron doses (especially of 0.5 MeV)?

J.J. BROERSE: It should be stated that our first results were partly based on macroscopic observations. Only recently the histological confirmation for all cases became available. In a number of animals small tumours were observed at necropsy, which increased the level of prevalence. As you can see from Fig.6 of our paper, for some situations we shall be able to derive RBE values for lower levels of prevalence. For these situations the RBE will generally increase with decreasing prevalence (i.e. with decreasing dose). The lowest doses employed up to the present are 2.4 and 5 rads for irradiations with mono-energetic neutrons with energies of 0.5, 4 and 15 MeV respectively. Recently we have started fractionated irradiations that will provide additional information about the risks of repeated low-level exposure.

H. WIJCKER: You have reported a lot of experimental data here. Can the results obtained be explained by some biological mechanism – albeit only qualitatively – in particular the influence of hormone application on radiation effects?

J.J. BROERSE: Our studies clearly indicate that the process of mammary carcinogenesis is hormone-dependent (see the effect of hysterectomy and of hormone administration). In the animals treated with oestrogen we observed a shorter induction time, and an increased incidence and increased multiplicity of the mammary tumours. In co-operation with the group of V.D. Mole and Blankenstein of the Rotterdam Medical Faculty we are currently investigating the possible relation between increased hormone levels in the blood plasma, the secretion of prolactin by the pituitary and the presence of oestrogen receptors in the rat mammary tissue.

M. DELPLA: If I have understood you correctly, the lowest neutron dose used is 5 rads but, if one takes the RBE into account, this will be equivalent to at least 100 rem (or 1 Sv). In the group that received a 5-rad neutron dose not a single tumour was observed, i.e. much less than in the control group.

Have you taken this result into consideration and, if so, did you find that the reduction in tumour frequency was statistically significant?

J.J. BROERSE: For the irradiation of Sprague-Dawley rats with 0.5 MeV neutrons we observed an RBE of 20 at a cumulative prevalence level of 30%

(5-rad neutron dose). The concept of dose equivalent is only intended to be used for radiation protection applications in man. We also have to assess the RBE values for the other strains before any extrapolation can be made with regard to the quality factor, Q, for humans.

The cumulative tumour prevalence was scored at a time when the number of spontaneously occurring tumours was still very low or zero. At dose levels of 5 rads of 15 MeV neutrons (Sprague-Dawley) and 5 rads of 0.5 MeV neutrons (WAG/Rij) the cumulative prevalence values are 30%, which are significantly higher than the prevalence level observed in the controls at the age of 22 months.

RADIATION-INDUCED TUMOURS IN C57BLf/6JNrs[SPF] AND C3Hf/HeMsNrs[SPF] STRAIN MALE MICE

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Abstract

RADIATION-INDUCED TUMOURS IN C57BLf/6JNrs [SPF] AND C3Hf/HeMsNrs[SPF] STRAIN MALE MICE.

Mice at the age of 12 weeks were irradiated with single graded doses of gamma rays delivered from caesium-137. The mice were kept in specific pathogen-free (SPF) conditions until death. All the mice were autopsied and examined histologically. In this communication, autopsy data from 385 males of C57BLf/6JNrs[SPF] and 278 males of C3Hf/HeMsNrs[SPF] mice are summarized. The median survival time of unirradiated control mice was 29 months for the C57BL and 25 months for the C3H mice respectively. The incidence of tumour-bearing mice in the control groups was 71.3% for the C57BL and 90.9% for the C3H mice. Major, spontaneous tumour types were reticular cell sarcoma (51.3%), liver tumour (8.8%), lung tumour (11.3%) for the C57BL, and liver tumour (84.6%), lung tumour (8.2%) and non-thymic lymphoma (3.6%) for the C3H mice. Miscellaneous tumours with a low incidence were vascular, bone, muscle, adrenal tumours and others. In the C57BL mice the incidence of reticular cell sarcoma declined gradually with increasing doses of radiation exposure from 0 to 800 R. Histological examination revealed that reticular cell sarcomas normally found in unirradiated C57BL mice originated from abdominal lymphatic tissues whereas lymphoblastic lymphoma in irradiated mice arose from thymus and/or submandibular lymph nodes. It is noteworthy that the peak incidence of thymoma (33.3%) was found after whole-body exposure up to 700 R. Myeloid leukaemia was also induced although to a slight extent. The age at death with lymphoreticular tumours and myeloid leukaemias was shortened in a dose-dependent manner. In the C3H mice tumour induction by radiation was generally not remarkable. The incidence of myeloid leukaemia attained a peak (15%) at 200 R. A lowering of the age at death was found to be proportional to the dose delivered. Lymphoreticular tumours were not increased with doses of radiation. A high background incidence of liver tumour of this strain was not affected until 400 R; however, it can be noted that some liver tumours of irradiated mice were hepato-cholangiocarcinoma with a very high rate of lung metastasis.

1. INTRODUCTION

Several years ago we initiated a research project on radiation carcinogenesis using specific-pathogen-free (SPF) mice with emphasis on the genetic influence on the incidence and types of naturally occurring as well as radiation-induced tumours. In this study, we first employed C57BLf/6JNrs[SPF] and C3Hf/HeMsNrs[SPF] strains. The reasons for selecting these two strains are as follows: (a) C57BL/6J and C3H/He are the most commonly used laboratory mouse strains throughout the world, and are characterized by different naturally occurring tumour types and incidences [1]. (b) These strains are characterized by different H-2 haplotypes, H-2^b and H-2^k, respectively. It is well known that the genes located in the H-2 complex exert a critical influence on the susceptibility of the host to a variety of oncogenic viruses [2]. (c) We know that T-cell-dependent immune functions, which are assumed to have a vital role in the immunological surveillance against tumour development [3], decline rapidly in the first strain, whereas they decay rather slowly in the second strain [4]. The reasons for using SPF mice for our purpose are obvious. It is known that the degree of infection, or the amount of non-pathogenic microbes in the environment, can greatly influence the incidence of myeloid leukaemias induced by radiation in RFM mice [5]. In this communication, we wish to summarize our preliminary data on the types and incidence of tumours developed in irradiated and unirradiated male mice of these two strains.

2. MATERIAL AND METHODS

Mice used in the present study included 385 males of the C57BLf/6Nrs[SPF] and 278 males of the C3Hf/HeMsNrs[SPF] strains, both bred in the Animal Production Facility of our institute. All mice, both experimental and unirradiated control, were allowed to live their entire life spans within the barrier-sustained SPF facility. For irradiation, the mice were irradiated at the age of 12 weeks with single graded doses of gamma rays delivered from 5000 Ci of ¹³⁷Cs placed within the SPF facility at a dose-rate between 98.5 and 101 rads/min. The mice were placed within an acrylic plastic box which was allowed to rotate during radiation exposure. After death, all mice were autopsied and examined histologically. The specimens were fixed with 10% phosphate-buffered formalin solution, pH 7.0, and stained with haematoxylineosin routinely, with PAS stain used only occasionally.

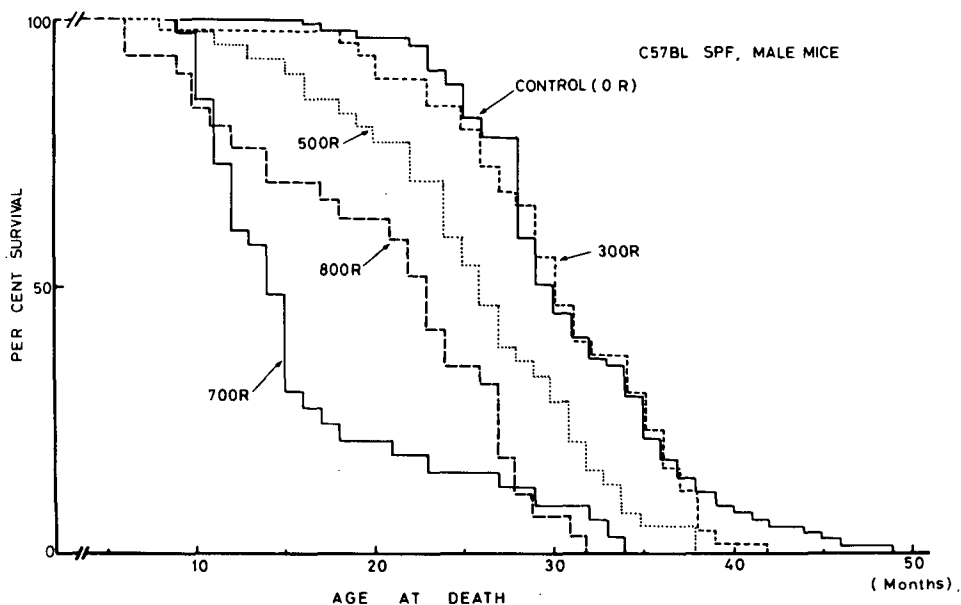


FIG.1. Survival curves for unirradiated and irradiated groups in male C57BL/6-SPF mice.

3. RESULTS

3.1. Tumour development in C57BL/6-SPF

3.1.1. Unirradiated control mice

Survival curves of irradiated as well as unirradiated C57BL mice are shown in Fig.1. The survival curve obtained with control mice gave a 50% survival time of 29 months. The types and incidences of naturally occurring tumours in control mice are shown in the first column of Table I. It can be seen from this table that tumours were found in 57 out of 80 unirradiated mice (71.3%). The total number of tumours recovered from 57 tumour-bearing mice was 66, indicating that the number of tumours per tumour-bearing mouse was 1.16. Most tumours were reticular cell sarcoma (41/66, or 62.1%), which is one of the most typical lymphoreticular tumours observed in mice. These tumours developed mainly from the liver (26/41, or 63.4%), some from the mesenteric lymph nodes (13/41, or 31.7%) as well as from the spleen (2/41, or 4.9%). One of the reticular cell sarcomas originating from mesenteric lymph nodes showed a histological similarity to Hodgkin's disease with a marked pleomorphism. One of the 7 liver cell tumours was a hepatocellular carcinoma with lung metastasis. Two of the 9 lung tumours

TABLE I. HISTOLOGY AND INCIDENCE OF RADIATION-INDUCED TUMOURS IN C57BL/6-SPF MALE MICE

	Control (%)	100–400 R (%)	500–800 R (%)
No. of mice	80	162	143
No. of tumour-bearing mice	57 (71.3)	111 (68.5)	84 (58.7)
<i>Histology</i>			
Lymphoreticular tumours:	41 (51.3)	71 (43.8)	57 (39.9)
Thymic lymphoma	0	2 (1.3) ^a	19 (13.3) ^a
Nonthymic lymphoma	0	13 (8.0)	15 (10.5)
Reticular cell sarcoma	41 (51.3) ^b	56 (34.6) ^{b,c}	23 (16.1) ^c
Myeloid leukaemia	0	6 (3.7)	4 (2.8)
Liver tumour	7 (8.8)	23 (14.2)	8 (5.6)
Lung tumour	9 (11.3)	23 (14.2)	11 (7.7)
Renal tumour	0	2 (1.2)	1 (0.7)
Adrenal tumour	0	2 (1.2)	2 (1.4)
Testicular tumour	0	0	1 (0.7)
Skin tumour	0	2 (1.2)	1 (0.7)
Muscle tumour	0	1 (0.6)	1 (0.7)
Haemangioma	6 (7.5)	11 (6.8)	5 (3.5)
Bone tumour	2 (2.5)	0	3 (2.1)
Gastric tumour	0	0	1 (0.7)
Total No. of tumours in tumour-bearing mice	66	141	96

^a $p < 0.001$, ^b $p < 0.025$, ^c $p < 0.001$.

were pulmonary adenocarcinoma with epidermoid pattern. Cavernous haemangiomas were observed in 2 cases in the spleen and in 4 cases in the liver. No myeloid leukaemia was found. No amyloidosis was found in any organs in all mice examined.

3.1.2. Irradiated mice

Survival curves of irradiated C57BL/6-SPF mice shown in Fig.1 indicate two interesting features: Firstly, there was no evidence of life-shortening in mice

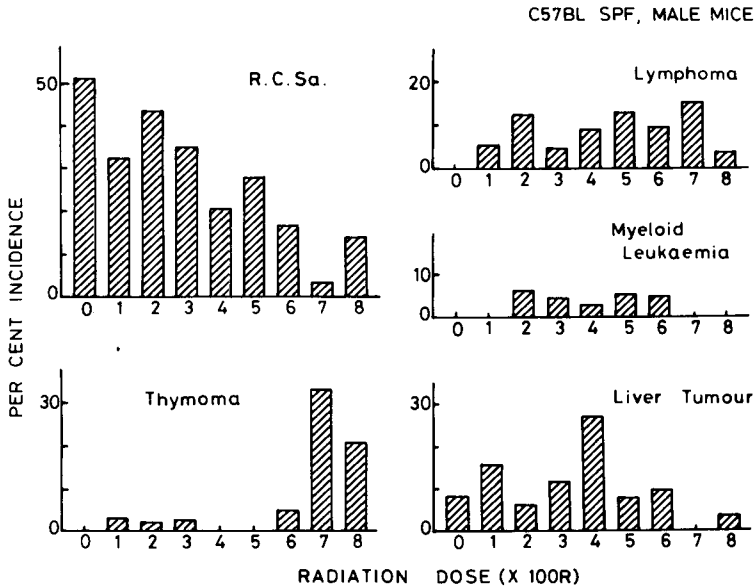


FIG.2. The incidence (%) of lymphoreticular tumours, myeloid leukaemias and liver tumours in male C57BL/6-SPF mice exposed to various doses of gamma rays at the age of 12 weeks.

irradiated with doses below 300 R. Secondly, the 50% survival time of 700 R-irradiated mice was significantly shorter than that of the 800 R-treated group. The incidence of tumours developed in different irradiation groups is shown in Fig.2. As can be seen from this figure, the incidence of reticular cell sarcomas appeared to decrease gradually with increasing doses of radiation exposure from 0 to 800 R. In contrast, there was a significant increase in the number of lymphoblastic and lymphocytic lymphoma arising mainly in the lymph nodes of the submandibular region in the irradiated group that was independent of the radiation dose. It is interesting to note that the peak incidence of thymic lymphoma of the lymphoblastic type with numerous starry-sky patterns (33.3%) was found after a single whole-body exposure to 700 R. Thymic lymphoma with enlargement of other lymphatic tissues was rarely seen in C57BL/6-SPF mice. The incidence of non-thymic lymphomas moderately increased following exposure to doses between 200 and 700 R, and then decreased after exposure to 800 R. Myeloid leukaemia was also increased, although not so markedly. The ages at death with the lymphoreticular tumours (Fig.3) and myeloid leukaemias were lowered in a dose-dependent manner. The incidence of hepatic tumours in the irradiated group, with a peak incidence (26.5%) in the 400 R-treated group,

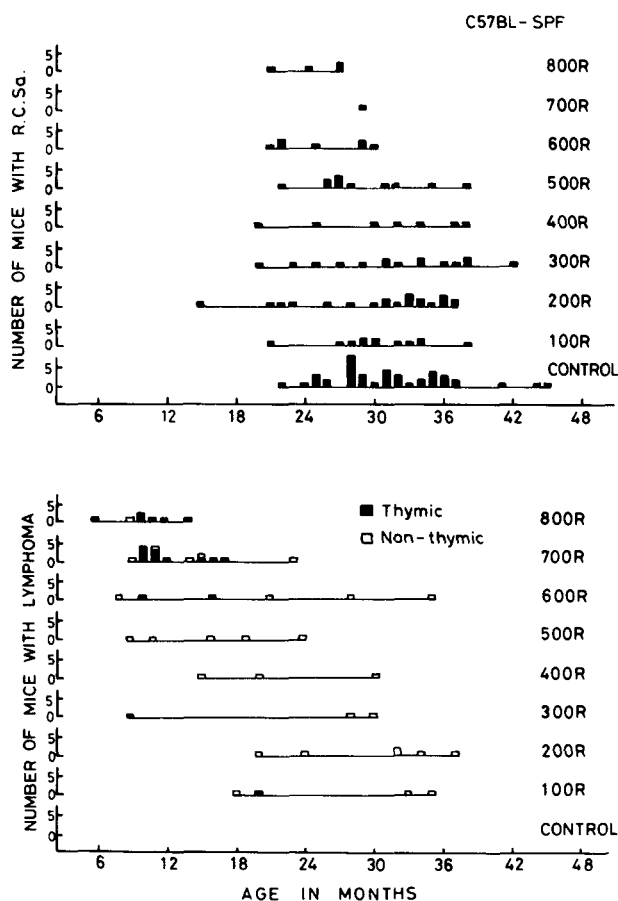


FIG.3. Distribution of age at death with reticular cell sarcoma (top), and thymic and non-thymic lymphomas (bottom) in male C57BL/6-SPF mice irradiated with single graded doses of gamma rays.

was higher than that in the control group (Fig.2). In some cases, hepatocellular carcinomas with lung metastasis were found. The incidence of lung tumour was slightly higher in mice exposed to relatively low doses (100–400 R), but not in those irradiated with higher doses (500–800 R) (Table I). Miscellaneous tumours, e.g. renal, gastric, adrenal, testicular, skin, muscle, vascular and bone origins, were also induced in groups exposed to higher doses of radiation (Table I). No amyloidosis was found in any of these groups.

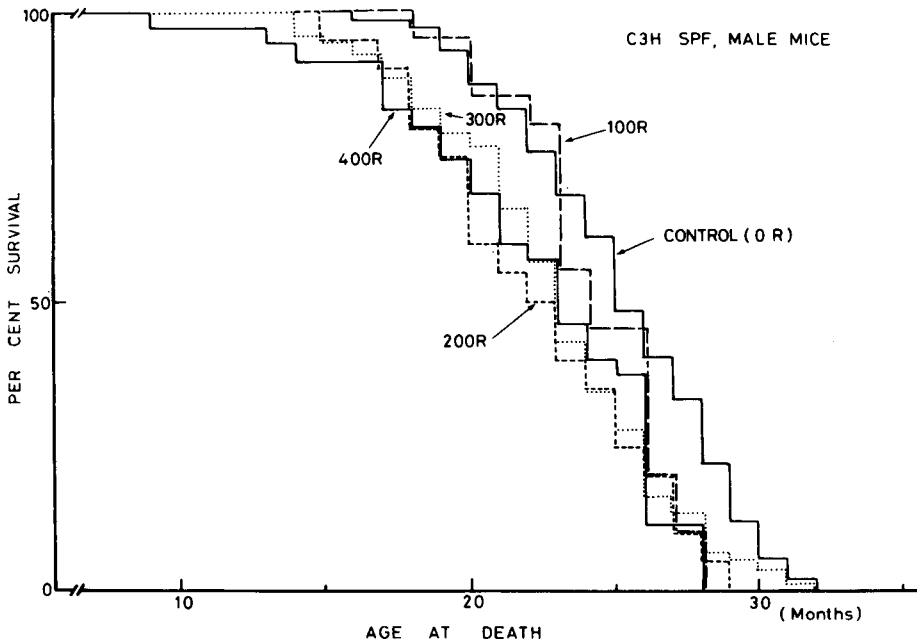


FIG. 4. Survival curves for unirradiated and irradiated groups in male C3H/He-SPF mice.

3.2. Tumour development in C3H/He-SPF mice

3.2.1. Unirradiated control mice

Survival curves of the unirradiated as well as the irradiated C3H/He mice are shown in Fig. 4. It is evident from this figure that the 50% survival time of control mice was 25 months. The types and incidences of tumours developed in the control mice are summarized in Table II. The tumour incidence observed in unirradiated control mice was 90.9%, or 100/110. The total number of tumours that developed in 100 tumour-bearing mice was 113, indicating that the average number of tumours per tumour-bearing mouse was 1.13. It is evident from this table that most of the tumours in these mice were hepatic tumours (93/113, or 82.3%) including 4 cases of metastasizing liver tumours. The other tumours observed were lung tumour, non-thymic lymphoma, renal tumour, adrenal tumour, muscle tumour and haemangioma of the liver. Neither thymic lymphoma, reticulum cell sarcoma, myeloid leukaemia, nor amyloidosis was found in this strain.

TABLE II. HISTOLOGY AND INCIDENCE OF RADIATION-INDUCED CANCER IN C3H/He-SPF MALE MICE

	Control mice (%)	100–400 R (%)
No. of mice	110	168
No. of tumour-bearing mice	100 (90.0)	160 (95.2)
<i>Histology</i>		
Lymphoreticular tumours:	4 (3.6)	9 (5.4)
Thymic lymphoma	0	1 (0.6)
Nonthymic lymphoma	4 (3.6)	6 (3.6)
Reticular cell sarcoma	0	2 (1.2)
Myeloid leukaemia	0 ^a	14 (8.3) ^a
Liver tumour	93 (84.6)	142 (84.5)
Metastasizing liver tumour	4 (4.3) ^b	19 (13.4) ^b
Lung tumour	9 (8.2)	11 (6.5)
Renal tumour	1 (0.9)	0
Adrenal tumour	1 (0.9)	6 (3.6)
Testicular tumour	0	1 (0.6)
Skin tumour	0	3 (1.8)
Muscle tumour	2 (1.8)	4 (2.4)
Haemangioma	3 (2.7)	1 (0.6)
Bone tumour	0	1 (0.6)
Salivary gland tumour	0	2 (1.2)
Total No. of tumours in tumour-bearing mice	113	194

^a $p < 0.005$, ^b $p < 0.025$.

3.2.2. Irradiated mice

The 50% survival times of mice irradiated with doses ranging from 100 to 400 R were similar (23 months) and were slightly shorter than those of unirradiated C3H/He-SPF mice. The incidence of tumours recovered from irradiated mice is

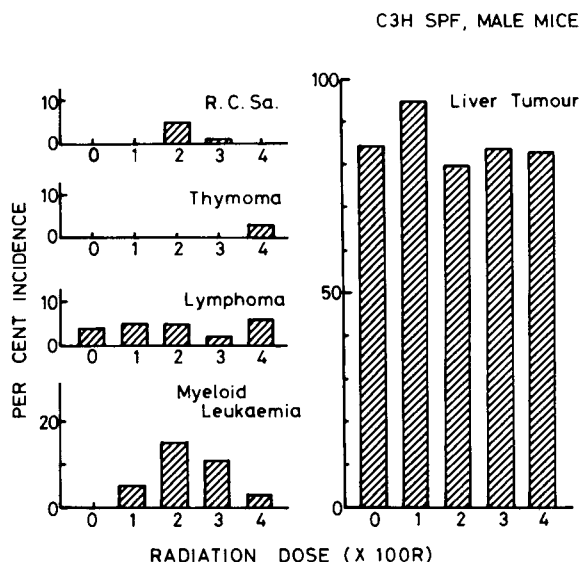


FIG.5. The incidence (%) of lymphoreticular tumours, myeloid leukaemias and liver tumours in male C3H/He-SPF mice exposed to various doses of gamma rays at the age of 12 weeks.

summarized in Fig.5. It is evident from this figure that the incidence of hepatic tumours, which characterized the spontaneous tumours of this strain, did not increase following irradiation. However, it was noted that 19 out of 142 hepatic tumours recovered from irradiated mice were hepatic tumours with pulmonary metastasis (13.4%) (Table II). The 12 out of 19 hepatic tumours with metastasis (63.2%) were of the mixed type, hepatocholangiocarcinoma. The remaining 7 cases of the metastasizing hepatic tumours consisted of a cholangiocarcinoma with metastases to both hepatic hilar lymph nodes and lungs (5.8%), and 5 cases of islet type (26.3%) and a diffuse type (5.8%) of hepatocellular carcinomas.

The overall incidence of myeloid leukaemia was 8.3% (Table II), which was statistically significant ($p < 0.005$) when compared with that of unirradiated control mice. The age at death of mice with myeloid leukaemia was lowered in a dose-dependent manner. Although the incidence of lymphomas was not increased after irradiation, one thymic lymphoma was found in 400 R-treated mice (2.9%). The incidence of lung tumour did not increase in the irradiated groups. Miscellaneous tumours, e.g. adrenal, muscle, skin, salivary gland, testicular and others, were definitely increased in mice irradiated with 200 to 400 R.

4. DISCUSSION

4.1. Lymphoreticular tumours

It has been known for some time that lymphoreticular tumours develop frequently in C57BL/6 and C57BL/ka mice [6–8], and that the major type of these tumours is reticular cell sarcoma which is generally called a histiocytic type or type A in Dunn's criteria [6]. In the present study, the reticular cell sarcomas observed in C57BL/6-SPF mice mainly arose from lymphatic tissues of the liver and mesenteric lymph nodes. Following irradiation, the incidence of this tumour declined with increasing doses of radiation. In contrast, the incidence of non-thymic lymphoma increased slightly following irradiation and this increase was not dependent on exposure doses ranging from 200 to 700 R. However, the incidence of thymic lymphoma increased greatly in mice exposed to a single whole-body dose of 700 R. Similar results had been reported by Furth and co-workers in LAF₁ male mice as early as 1959 [9]. These results compare with earlier observations of Kaplan and co-workers [10–12] that the incidence of thymic lymphoma increased specifically when C57BL mice were treated with four, weekly fractionated, whole-body exposures to 170 R each, or a total dose of 680 R. Thus it appears that the fractionation regimen routinely used for induction of thymomas in C57BL mice may not be critical for pathogenesis of this tumour, and that the total dose is the most crucial, provided that early mortality would not result as in the SPF mice used in the present study. It was also noted that the types of lymphoreticular tumours developed in irradiated mice appeared to shift dose-dependently from late occurring abdominal lymphatic tumours to the thymic lymphoma which are relatively early occurring tumours in irradiated mice. The relationship between the peak incidence, types of lymphoreticular tumours and ages at death, shown in Table I and Figs 2 and 3, suggests strongly that lymphoblastic lymphomas arising from the lymph nodes of the sub-mandibular region represented the transitional form of the two types of lymphomas discussed above. No reticular cell sarcoma was observed in unirradiated C3H/He-SPF male mice.

4.2. Myeloid leukaemia

No myeloid leukaemia has been found so far in the unirradiated male mice of both the C57BL/6-SPF and C3H/He-SPF strains, but its incidence apparently increased following irradiation. Although the increase in the incidence of myeloid leukaemia did not appear to be dose dependent in C57BL/6-SPF mice (Fig.2), its dose dependence was clearly seen in C3H/He-SPF mice (Fig.5). The peak incidence was observed in the 200 R-treated group, in contrast to the one reported by Upton and co-workers [13] in male RF/Un mice, in which the peak incidence

was observed in the 300 rads-irradiated group. All the myeloid leukaemias induced in C3H/He-SPF mice were chloroleukaemia (granulocytic type), and no erythrocytic leukaemia was found. Ages at death of induced leukaemias in both strains were lowered with increasing doses of radiation.

4.3. Hepatic tumours

In C57BL/6-SPF mice, the incidence of hepatic tumours apparently increased following exposure to 100–400 R, and the incidence observed at 400 R (26.5%) was statistically significant ($p < 0.025$). At these dose levels, the incidence of hepatic tumours did not appear to compete with the thymic lymphomas which developed earlier in mice exposed to 700 R. A few adenocarcinoma with lung metastasis were found.

In C3H/He-SPF mice, most tumours developed were liver tumours and their high incidence was almost the same for all irradiated and control groups. We might add, however, that no special attempt was made to control the bedding material which is known to alter the natural incidence of this tumour in C3H-A^{vy} mice [14]. It is noteworthy that the incidence of metastatic liver tumours was significantly increased in the irradiated group ($p < 0.025$). The histological type of most of the metastasizing liver tumours in both control and irradiated groups was hepato-cholangiocarcinoma.

5. CONCLUSION

The results reported in this communication point to a theory that genetic factors exert a crucial influence on the types and incidence of naturally occurring as well as radiation-induced tumours in mice. We are now in the process of extending these studies to other strains with H-2 haplotypes different from those of the two strains employed in the present study. We believe that experiments of this nature will contribute much to our understanding of the mechanisms of tumorigenesis, in particular of radiation carcinogenesis.

ACKNOWLEDGEMENT

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DISCUSSION

W. GÖSSNER: Could you please give us more information about your criteria for the diagnosis of the 123 non-metastasizing liver tumours you observed. I am asking this question because there is still some controversy about the exact definition of liver tumours, especially in mice. In particular there is some difficulty in distinguishing between hyperplastic and neoplastic liver nodules, and between benign and malignant neoplastic lesions in the mouse liver.

T. SADO: I am afraid I cannot answer that particular question because I did not perform the tumour diagnoses myself. All I can say at this moment is that hepatic tumours which metastasized to other sites, in particular to the lung, were considered as malignant hepatic tumours, and those which did not metastasize were considered as non-malignant, or benign tumours.

I might add that, as I understood from discussion with Mr. Kasuga who did the pathology, most of the liver tumours that developed in C3Hf mice were derived from hepatic cells, whereas tumours that developed in C57BLf mice were diffuse-type reticular cell sarcomas originating from RES of the liver. The latter type of tumours were not included in the category of hepatic or liver tumours.

ANALYTICAL APPROACHES TO AND INTERPRETATIONS OF DATA ON TIME, RATE AND CAUSE OF DEATH OF MICE EXPOSED TO EXTERNAL GAMMA IRRADIATION*

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Abstract

ANALYTICAL APPROACHES TO AND INTERPRETATIONS OF DATA ON TIME, RATE AND CAUSE OF DEATH OF MICE EXPOSED TO EXTERNAL GAMMA IRRADIATION.

Young adult male and female mice of inbred strains, A, BALB/c, C57BL/6 and C57L, and B6CF₁ and F₂ hybrids were exposed to daily duration-of-life external ⁶⁰Co gamma irradiation. Age at death was recorded, and most of the deceased were necropsied to ascertain occurrence of major types of tumours. Age- and cause-specific mortality or incidence rates were derived, and their regressions on age were fitted with polynomial equations by least-squares procedures. Age-specific and age-adjusted integrated lifetime risk in excess of the control population was expressed as the mortality ratio (irradiated/control). Linear and nonlinear functions and widely different life expectancies can be accommodated by this technique. These basic actuarial statistics provide a means for comparative analysis of dose-response functions, sex and genetic variables, relative versus absolute risk, protraction or dose-rate factors and major contributing causes of excess risk. They also provide a basis for extrapolation to man. As examples, life shortening in days per rads (4 days/100 rads accumulated) is generally independent of sex, genotype and daily dose rate. The integrated average lifetime risk of death related to all tumours (0.025%/rad) is largely independent of sex, genotype and dose-rates <12 rads/day, despite the fact that tumour incidence varies by a factor of 2 to 3 among genotypes. At low exposure rates, tumour-related mortality accounts for 80% of the excess risk, and life shortening is a function only of accumulated dose, independent of dose rate below 12 rads/day. The radiobiological effectiveness for low daily exposure levels is less than that for single exposures by a factor of 5 to 10. Life shortening following low daily exposure rates is induced at the rate of 0.03–0.06 days/R for the mouse, which extrapolates to about 1–2 days/R for man.

* Work supported by the United States Department of Energy.

INTRODUCTION

The questions remaining to be answered in the field of applied radiobiology are the motivation for this symposium. Two of the questions of paramount concern have interested us for many years:

1. What are the dose-response functions for the important pathological sequelae of man's exposure to continuous low intensities of external radiation for major portions of the lifetime?
2. How can we best employ and exploit the extensive data from the exposure of experimental animals for extrapolation to man, especially for those issues wherein human statistics are particularly inadequate?

These and similar questions have stood for an embarrassingly long time, even though many of us were confident they should be soon answered, as they did not lack for attention. Any complete annotation of the efforts of individuals and of research groups, let alone national and international committees, that have concerned themselves with these issues would require an historical compendium. The previous symposium of this series [1] is a partial catalogue, to which one can add the recent efforts of the United Nations [2], the U. S. National Research Council - National Academy of Sciences [3], and a small but comprehensive review held in Chicago [4].

The active research groups have been characterized by their selective interest, usually in only one species and in limited biological end points, methods of exposure and analytical tools. There is little consistency beyond the use of dogs and mice for most work. Before the appearance of the JANUS biomedical research reactor [5] on our experimental scene, our interests at Argonne focused on lifetime exposures, life shortening and life tables, with the mouse as species of choice.

The rationale was straightforward then and still is. The mouse is economical to use and has many attributes in common with man. The duration-of-life exposure regime (usually commencing at young adulthood) most nearly duplicates any anticipated excess exposure of the general population or the work force. The end point is unequivocal, and the analytical approach recognizes the underlying mixture of predetermined and random forces of mortality characteristic of all mammals.

The basic methods were presented by Sacher in 1950 [6] and by Brues and Sacher in 1952 [7]. They were further developed and applied to radiation lethality data by Sacher [8,9,10] and Grahn [11,12], and also to the consideration of other problems in natural aging [13,14]. We have also extended the analysis to specific causes of death, or to lesions associated with the event of death [7,11,12]. Many features of the experimental data have been summarized in the referenced reports, or in others to be noted. However, there are special analytical applications that have not yet been fully documented, and our unique body of data, from more than twenty years of research, can continue to be used to demonstrate basic approaches to the analysis of present-day problems in environmental toxicology. We also continue to believe that our basic methods offer a comprehensive approach to the questions stated above.

MATERIALS AND METHODS

We employed a variety of inbred strains of laboratory mice and selected F₁ and F₂ hybrids. The inbreds were the strains A/J, BALB/cJ, C3Hf/He,

C57BL/6J and C57L; the hybrids were the LAF₁ (C57L x A/J), B6CF₁ (C57BL/6J x BALB/cJ) and the B6CF₂. Generally, both sexes were used in equal numbers. The mice were irradiated beginning at about 100 days of age.

The mice were placed three to a cage at an assigned position in the low-intensity ⁶⁰Co gamma radiation facility [15] where they received a specified daily exposure until death. Exposures of 8 to 12 hours duration took place during the night so that routine animal care and observation could be provided during the normal work day, and so that the basically nocturnal mice would be exposed during their normal 'work day'.

Animals were housed in one-liter cylindrical transparent bakelite cages offering free access to food and water, and with wood shavings as litter. Controls were similarly housed and kept in the shielded entry maze of the radiation facility.

The daily dose levels of concern for this discussion were 0, 0.3, 1.3, 2.6, 5/6, 12, 24, 32, 43 and 56 roentgens per day. Dose rates in R/min would change as exposure time was adjusted for source decay, but on the average they were equal to $\frac{R/\text{day}}{600 \text{ min/day}}$. Thus, the rates ranged between 0.5 and 95 mR/min. The roentgen-to-rad conversion factor for these experimental conditions is 0.90 [16].

Day of death was determined precisely, and 70% to 90% of the mice were necropsied to obtain the incidence of the major neoplastic, degenerative and infectious diseases. Only about one-half of those autopsied underwent microscopic study. The identified causes of death include tumors of the reticular tissues, primary pulmonary tumors, ovarian tumors and cysts, hepatomas and degenerative renal lesions. In addition, a miscellaneous group of tumors, including those of the mammary, adrenal and connective tissues were added when defining the 'deaths from all tumors' category. (The actual cause of death is often difficult to ascertain in the mouse, and our delineation of the age-specific and cause-specific mortality rates is shorthand for a more correct term 'age-specific mortality with the lesion of interest'.)

The mean after-survival (MAS) data, from the day of initial exposure to death, and derived for each sex, strain and daily dose group provide the initial analysis and a first basis for extrapolation to man. Age-specific death rates are a focal point of the analyses, however. Populations of mammals die at an exponentially increasing rate with increasing age after the juvenile period. Because the rate is constantly changing, it is ideally estimated over only brief time periods, so that

$$r_t = \frac{D_t}{N_t}$$

where r_t is the death rate for time interval t and D_t is the number of deaths occurring in the interval, and N_t is the number of animals alive at the beginning of the time interval. For small intervals, this reduces to the classical expression of death rate given by many authors [11,17,18],

$$r_t = \frac{1}{N_t} \frac{dN}{dt}$$

For practical calculations, r_t can be approximated by the expression

$$r_t = (\ln N_t - \ln N_{t+d})/d$$

TABLE I. COEFFICIENTS FOR THE REGRESSION OF \ln MEAN AFTER-SURVIVAL (MAS) ON EXPOSURE IN ROENTGENS PER DAY

Strain or hybrid	Sex	Experiment 1		Experiment 2	
		Control MAS \pm SE (days)	$\beta \pm$ SE (Fraction lost per R/day)	Control MAS \pm SE (days)	$\beta \pm$ SE (Fraction lost per R/day)
A/J	♂	458 \pm 20	-0.038 \pm 0.002	} 502 \pm 12	-0.036 \pm 0.002
	♀	487 \pm 20	-0.038 \pm 0.002		
BALB/c	♂	387 \pm 21	-0.040 \pm 0.001	} 609 \pm 14	-0.042 \pm 0.001
	♀	453 \pm 19	-0.041 \pm 0.002		
C3Hf/He	♂	399 \pm 22	-0.035 \pm 0.001		
	♀	423 \pm 21	-0.036 \pm 0.002		
C57BL/6	♂	576 \pm 26	-0.038 \pm 0.002	} 645 \pm 14	-0.038 \pm 0.001
	♀	584 \pm 21	-0.038 \pm 0.001		
C57L	♂			} 534 \pm 10	-0.028 \pm 0.001
	♀				
B6CF ₁	♂	759 \pm 26	-0.033 \pm 0.001	} 753 \pm 10	-0.037 \pm 0.001
	♀	782 \pm 26	-0.033 \pm 0.001		
B6CF ₂	♂	548 \pm 27	-0.036 \pm 0.001		
	♀	561 \pm 24	-0.037 \pm 0.001		
Mean: (per R/day)			-0.037		-0.036
(per rad/day)					-0.04

where d is the number of days in the interval $[9,10]$. The array of age-specific death rates when plotted as $\ln r_t$ vs age generally conforms to a positive linear function that can be routinely fitted by least-squares regression techniques to yield the constants r_0 and k in the exponential equation,

$$r_t = r_0 e^{kt}$$

the Gompertz equation [9,18,19]. Although r_0 is algebraically the intercept at zero age, we have usually set this intercept at 100 days of age, the time of first exposure. In addition, 100 days of age is the approximate time of minimum death rate in the population, or cohort.

The death rates can also be calculated for any specific cause of death, rather than all causes, in which case the equation becomes [11]

$$r_{ts} = \ln \left[1 - \frac{n_s}{N_t} \right]^{-1} / t$$

where n_s is the number dying of the specific cause in age interval t . The cohort or dose group can also be decremented for any specific cause by eliminating those cases from the number dying and the number at risk at the beginning of the age interval in which the specified deaths occur. This is an especially useful procedure for removal of deaths that fall in a distinctly nonlinear pattern, such as those from reticular tissue tumors, for example, thus permitting a more rigorous linear analysis of the death rate regressions [11,12].

Finally, the display of age-specific death rates versus age, on the semilog plot, may often be nonlinear. As noted, leukemia mortality is characteristically phasic and therefore nonlinear. Other tumors, lesions, dose groups or genetic strains may also have nonlinear characteristics. Since we wish to estimate quantitatively the effects of lifetime irradiation, our approach is to derive the least-squares fitted polynomial equation that minimizes the variance. Measurements of excess risk, or mortality in excess of the control, can then be obtained, regardless of the form of the curves, by integrating the area between the control and experimental curves and reducing this value to terms of unit time [11,12]. This procedure will be discussed in more detail in a later section. The resulting measure of average displacement of the irradiated population from the control is expressed as the logarithm of the standard mortality ratio commonly used in epidemiology. This is the ratio of observed mortality to the mortality expected in a standard population, which in our case is the control. A conceptually similar procedure has been used by Ullrich et al. [20,21] to adjust age-specific incidence data to the controls. The two procedures have not been concurrently evaluated for their consistency in deriving mortality ratios.

We have not yet developed standard errors for the estimated mortality ratios or displacements. Presumably they could be derived as a function of the errors of estimate associated with least-squares fitted age-specific mortality rate data. Standard errors that are given have been derived from variance analyses of the array of parameters developed for the dose by strain matrix.

RESULTS AND DISCUSSION

Mean After-Survival

One of the most consistent findings has been the observed exponential relationship between MAS and daily dose [4,15]. Table I presents the regression coefficients of the equation,

$$MAS_D = MAS_0 e^{-\beta D}$$

for the two sexes and diverse strains and hybrids. Genetic variation is characterized by differences in the zero-dose intercept, not by variation in slope. The mouse can therefore be characterized by a common slope value of -0.037 per R/day or about -0.04 /rad/day. This is equivalent to a loss of 4 days per 100 rads accumulated, which is one-fifth to one-tenth of the loss induced by single exposures [9,22].

TABLE II. BASIC STATISTICS FOR INDICATED MOUSE STRAINS AND EXPOSURE LEVELS.

Data for the two sexes combined regressions of \ln age-specific mortality rate on age (k) given for all causes of death fitted with least-squares linear equations

	Exposure (R/day)							Common intercept	Control tumor incidence at 800 days	
Item	0	1.3	2.6	6	12	24	32	(rate/day)	(rate/day)	(%)
<i>A/J</i>										
MAS ^a (days)	502	480	492	472	394	230	167	1.2 × 10 ⁻⁴	129 × 10 ⁻⁴	41.1
Mort. ratio	1.0	1.12	1.11	1.18	1.89	5.42	11.5			
k (× 10 ⁻³ /d)	7.20	7.51	7.52	7.74	9.62	19.50	31.63			
No. mice	151	155	91	90	87	89	44			
% with tumors ^b	41.1	36.8	42.9	46.7	39.1	30.3	22.7	8 × 10 ⁻⁶		
<i>BALB/c</i>										
MAS ^a (days)	609	567	509	499	382	230	162	5.5 × 10 ⁻⁵	41 × 10 ⁻⁴	45.0
Mort. ratio	1.0	1.37	2.04	2.23	4.35	9.97	20.1			
k (× 10 ⁻³ /d)	6.79	7.59	8.89	8.92	12.96	23.48	36.74			
No. mice	151	150	81	89	81	91	44			
% with tumors ^b	57.0	62.7	56.8	67.4	63.0	27.5	11.4	15 × 10 ⁻⁶		
<i>C57BL/6</i>										
MAS ^a (days)	645	602	573	580	476	263	194	5 × 10 ⁻⁵	8 × 10 ⁻⁴	21.5
Mort. ratio	1.0	1.24	1.57	1.58	2.79	10.9	14.3			
k (× 10 ⁻³ /d)	6.64	7.13	7.81	7.83	10.21	20.27	30.32			
No. mice	135	141	81	83	88	91	41			
% with tumors ^b	31.1	44.0	35.8	37.3	40.9	58.2	41.5	9 × 10 ⁻⁶		

<i>B6CF₁</i>									
MAS ^a (days)	753	705	677	590	457	291	210		
Mort. ratio	1.0	1.43	1.73	2.95	6.17	17.8	27.4		
k (X 10 ⁻³ /d)	7.13	7.83	8.32	9.92	13.45	21.52	33.60	1.4 X 10 ⁻⁵	
No. mice	303	301	181	182	89	87	44		
% with tumors ^b	62.7	78.4	76.8	74.7	62.9	52.9	65.9	5 X 10 ⁻⁶	13 X 10 ⁻⁴ 32.7

^a See Ref. [11] for complete presentation of data by sex and strain.

^b Percentage of all necropsied mice showing evidence of one or more tumors.

TABLE III. REGRESSIONS OF LOG MORTALITY RATIO (ln MR) ON ACCUMULATED DOSE (kilorontgens) AND RELATED PARAMETERS
See text for derivations and abbreviations

Exposure level (roentgens/day)											
Strain	1.3	2.6	6	12	Mean 1.3–12	LS ^a (d/R)	24	LS (d/R)	32	LS (d/R)	z ^a (days)
<i>Deaths from all causes (ln MR/kilorontgen)</i>											
A/J	0.11	0.06	0.04	0.10	0.079	0.011	0.26	0.04	0.38	0.05	140 ± 8
BALB/c	0.31	0.40	0.18	0.26	0.286	0.045	0.35	0.06	0.47	0.07	157 ± 5
C57BL/6	0.19	0.22	0.10	0.15	0.164	0.027	0.28	0.05	0.37	0.06	166 ± 4
B6CF ₁	0.27	0.23	0.23	0.26	0.247	0.041	0.30	0.05	0.41	0.07	167 ± 2
Mean ± SE	0.22	0.23	0.14	0.19	0.194 ±0.012		0.30 ±0.05		0.41 ±0.06		158 ± 6
LS (days/r)	0.034	0.036	0.022	0.030		0.031		0.047		0.064	
<i>Deaths with tumors (ln MR/kR)</i>											
A/J	-0.01	0.05	0.09	0.16	0.071		0.37		0.33		
BALB/c	0.06	0.40	0.30	0.26	0.254		0.27		0.34		
C57BL/6	0.69	0.37	0.17	0.23	0.364		0.54		0.49		
B6CF ₁	0.50	0.23	0.23	0.24	0.298		0.39		0.57		
Mean ± SE	0.31	0.26	0.20	0.22	0.247 ± 0.043		0.39 ± 0.05		0.43 ± 0.06		

<i>Deaths without tumors (ln MR/kR)</i>							
A/J	0.17	0.07	0.01	0.09	0.084	0.24	0.36
BALB/c	-0.09	0.20	0.01	0.10	0.056	0.29	0.40
C57BL/6	0.14	0.21	0.09	0.11	0.134	0.20	0.31
B6CF ₁	-0.05	0.02	0.12	0.24	0.082	0.24	0.29
Mean ± SE	0.04	0.13	0.06	0.13	0.089 ± 0.025	0.24 ± 0.02	0.34 ± 0.02

^a Derived from equation; days of life shortening (LS) = $z \ln MR/R$. See text for discussion.

If we assume that each species is characterized by its own life shortening coefficient that is independent of genetic variation, then one needs only this coefficient for man to predict his response to any low intensity continuous exposure to low-LET radiations. Can we derive an estimate from available data?

As data on the life-shortening effects of lifetime exposure of guinea pigs and dogs have shown [see 11,22], the life shortening coefficient per rad or roentgen increases as the species life expectancy increases. The increase over the mouse slope is roughly proportional to the ratio of life expectancies. If this holds for man, then the life-shortening coefficient for man should be approximately 30 times greater than that for the mouse. This estimate is based on a life expectancy for young U.S. adults at age 20 of about 20 000 days (55 years) [23]. While life expectancy for 100-day-old mice varies with strain (Table I), a figure of 1/30 of 20 000 or about 665 days is a reasonable value for the average mouse. The estimated life shortening coefficient for man is $(30)(0.04) = 1.2$ days/rad/day or 1.2 days per rad accumulated at the lowest intensities.

A note of caution is required. Although the data from the guinea pig and dog experiments generally support the above argument, there is no way to confirm the assumption directly for man. Sacher [13] cautions that, while the practice of species equalization by a transformation of the age scale has theoretical and experimental credibility, the procedure might be incorrect because the factor of 30 may not fully account for species differences in the intercept of the mortality rate slope. This method of transforming the age scale does need further evaluation. In the interim, we offer the life shortening coefficient of 1.2 days/rad, based on the approximate 30-fold ratio of MAS values from young adulthood, as one extrapolation to man.

Age-Specific Mortality Rate

Following single exposures, the death rate slope remains generally parallel to the control but is displaced upward [8,9,12]. Excess mortality is therefore easily defined by the displacement of the intercept of the regression of age-specific mortality rate on age (the Gompertz displacement defined by Sacher [8,9]). This displacement can be used to estimate the life-shortening coefficient in days per rad for mice (~ 26 to 44 d/R [9]), to compare species and to render extrapolations to man or other animals [9]. These general procedures and their application need no further elaboration for the case of single exposures, but they have not been fully explored for continuous exposures given over the adult life, although there is ample documentation that the effect of continuous exposure is manifest as a progressive divergence of the age-specific death rates from the control as age increases and dose accumulates [8,9,11,12].

For the sake of brevity, we will confine our analysis to the comparison of inbred strains A/J, BALB/c and C57BL/6, and the B6CF₁ hybrid. Data from the two sexes are combined. This does not introduce a bias, because they follow parallel responses, with the female generally showing higher incidences of tumors and slightly steeper regressions of mortality rate on age. The analysis is also restricted to exposures of 0, 1.3, 2.6, 6, 12, 24 and 32 R per day, starting at 100 days of age and continuing for the duration of life. The age-specific mortality rates were fitted with first or second degree polynomial equations through a common 100-day intercept for all exposure groups within each strain. The data are presented in three ways:

- 1) deaths from all causes;
- 2) deaths with one or more tumors;
- 3) deaths without evidence of a tumor.

The mean after-survivals and other pertinent results are given in Tables II and III.

The area between slopes, control versus experimental, as previously noted, is used to develop expressions of excess risk in terms of the mortality ratio. For derivations involving nonlinear equations, the area between the two curves, taken to the age interval of last death for the irradiated cohort and divided by the number of days between that age and 100 days, provides an estimate of the natural logarithm of the average lifetime mortality ratio, or $\ln MR$, for the specified daily exposure rate. The antilog gives the average lifetime ratio of observed over expected mortality or the factor of increase in death rate for that exposure cohort.

If the cumulative area is sequentially derived and divided by the number of days over which the area or displacement is calculated, a measure of $\ln MR$ is derived that is related to accumulating exposure in roentgens (or rads). Thus, one can calculate the coefficient of fractional increase in mortality ratio per roentgen, which, in turn, can be related to the reduction of life expectancy. This procedure can estimate the average life shortening coefficient for the specified cause of death. It is directly comparable to life shortening coefficients derived by Sacher from the Gompertz equations describing the response of mouse, rat, guinea pig and dog populations subjected to single exposures [9].

When all compared mortality-rate slopes are linear, the derivation reduces to manipulations of the increment of increase (or decrease) in slope. Thus:

$$\ln MR = [(k_D - k_0)]\Delta t/2$$

where k_D and k_0 are the Gompertz slopes for the irradiated and the control (fitted through a common intercept), and Δt is the lifespan (days from start of exposure to last death) of the irradiated group. The increase in average $\ln MR$ per R accumulated is:

$$\ln MR/R = \left[\frac{(k_D - k_0)/2}{R/\text{day}} \right]$$

A close relationship obviously must exist between the mortality ratio and the average life shortening (LS), so that

$$LS = z \ln MR$$

where z is the number of days lost per log cycle increase in the mortality ratio. The experimentally-derived value of z is between 140 and 170 days. A theoretical expectation of 164 days was given in a previous report [11]. The product of $(z)(\ln MR/R)$ provides a life-shortening coefficient in terms of days/ R . Figure 1 presents an example of the fitted mortality-rate data for the B6CF₁ hybrid used to derive the mortality ratios.

All Causes of Death

The regression coefficients of $\ln MR/R$ in Table III reveal little variation among the average slopes for the four exposure levels between 1.3 R/day and 12 R/day. According to a variance analysis, significant variation does

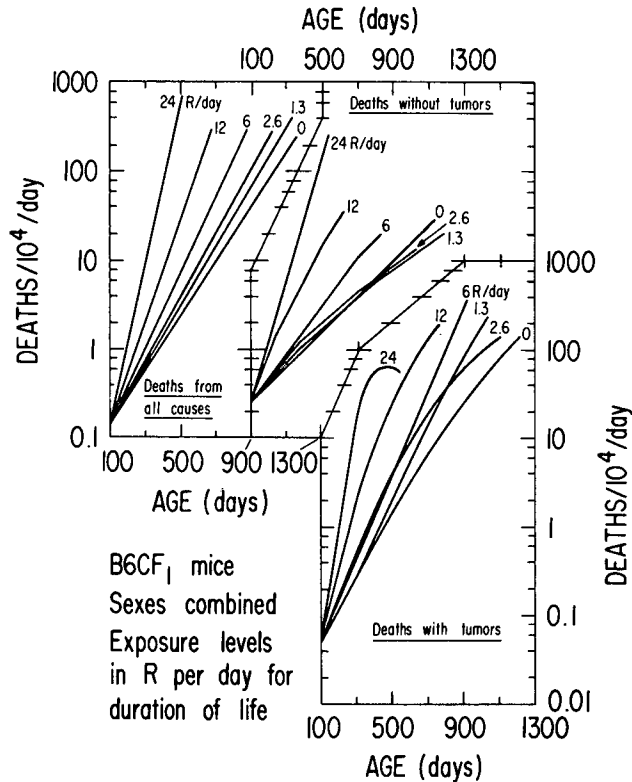


FIG.1. Regressions of death rate on age fitted by least-squares through a common intercept at 100 days. First or second degree polynomial equations.

exist among strains, but not among the four doses. If we include the data from 24 R/day in the analysis, then variation among the dose rate groups rises to the 5% level of statistical significance. Thus, we conclude that daily low intensity external γ -irradiation at 12 R/day and below induces excess mortality at a rate that depends only upon accumulated dose, and that is independent of daily or hourly exposure rate. The average slope is 0.19 ± 0.01 ($\times 10^{-3}$) per roentgen or 0.21×10^{-3} per rad. There is not much variation among the strains except for A/J which shows little radiation-induced excess mortality at these low-level exposures. This strain is characterized by a high incidence of death from chronic liver and kidney degeneration, an amyloid infiltration disease, that swamps the effects of low levels of radiation injury. The strain is included in this analysis, since it may represent a genetic variant of any mammalian population, including man.

Up to 12 R/day, life shortening from all causes of death averages 0.031 days per roentgen for these four dose rates and genetic groups, ranging between 0.011 to 0.045 days/R among strains, and between 0.022 and 0.036 among dose rates. Above 12 R/day, the life-shortening coefficient begins to rise rapidly, and the variance becomes more dependent upon dose rate than upon genetic factors.

TABLE IV. COMPARISON OF ABSOLUTE AND RELATIVE RISK ESTIMATES FOR DEATHS WITH ONE OR MORE TUMORS

Strain	Intercept at 100 days of age	Death rate per day at 500 days of age (400 days of exp.)					
		Excess death rate					
		0 R/d ($\times 10^{-6}$)	12 R/d ($\times 10^{-6}$)	($\times 10^{-6}$)	per R ($\times 10^{-8}$)	%/R	ln MR/R ($\times 10^{-3}$)
BALB/c	15×10^{-6}	373	4535	4162	87	0.23	0.26
C57BL/6	9×10^{-6}	117	1028	911	19	0.16	0.23
B6CF ₁	5×10^{-6}	155	2599	2444	51	0.33	0.24

All Deaths with Tumor

Since more than 50% of the mice die with evidence of one or more tumors (Table II), excess risk from tumor-related death closely matches that described for the category of all causes (Table III). The mortality ratios are greater, and the regressions of ln MR on accumulating exposure are steeper.

The variation among the four lowest exposure groups is not statistically significant and the average slope of ln MR on accumulating exposure is 0.25 ± 0.04 ($\times 10^{-3}$)/R (Table III). Again, the A/J strain diverges most from its peers. At the highest exposures of 24 and 32 R/day, the B6CF₁ and one of its parents, the C57BL/6 strain, reveal a somewhat higher than average rate of increase in excess mortality with tumors. This is due to sharp increases in the induction of reticular tissue tumors (leukemias) in these two strains.

One important finding emerges. Although sampling is limited, there is no significant correlation between the spontaneous incidence or rate of tumor mortality and the induced tumor mortality ratio per R. The induced rate per R per day is variable, however, and is positively correlated with the spontaneous rate. If the induced rate per R had been invariant, the mortality ratio would have been negatively correlated with spontaneous rate. In other words, the mortality ratio, or relative risk, is the less variable measure of determining excess tumor risk from low levels of radiation. Excess mortality with tumors occurs in a more nearly constant ratio to the spontaneous rate, rather than as a constant rate per rad per mouse-day.

An example may serve to clarify this observation. Table IV compares the two parent strains, BALB/c and C57BL/6, and their F₁, all of which have a generally uncomplicated response to radiation (as compared to strain A/J), at 0 R/day versus 12 R/day, and at the single time of 400 days after initiation of exposure. The log mortality ratio is nearly constant, but the increment of excess deaths varies from near 900 to over 4000 per million mice per day. The rate per total accumulated exposure ranges from 19 to 87

($\times 10^{-8}$) per R, with the F_1 intermediate between the two parents. The percentage increase per R only varies by a factor of two, and the hybrid has the highest rate of increase over its control. Although this limited comparison cannot settle the argument concerning the appropriateness of either relative risk or absolute risk for environmental impact assessment [3], it does add strength to the position that the relative risk assumption may be more correct for the usually uncharacterized and heterogeneous human population.

Deaths without Tumor

The mortality ratios in this residual category show a surprising degree of similarity among all four strains at the two highest exposure levels (Table III), although the incidence of nontumor deaths ranges between 30% and 90%. This may reflect the emerging importance of hematopoietic system injury at these highest exposures. In comparison, at the lower exposure levels, where nontumor deaths range more narrowly between 25% and 65% among the strains and doses, the data are extremely variable and no single source of variation is significant. The average rate of increase in $\ln MR/R$ is low, $0.09 \times 10^{-3}/R$, for all strains at 1.3-12 R/day. Since an average of 40% of the mortality is assigned to this category at these doses, the weighted contribution of nontumor deaths to the mortality ratio is $(.4)(.09 \times 10^{-3}) = .036 \times 10^{-3}$, as compared to those deaths with tumors, $(.6)(.25 \times 10^{-3}) = .15 \times 10^{-3}$. The sum, 0.186×10^{-3} , approximates the observed value for all causes, 0.19×10^{-3} . Thus, about 80% ($0.150/0.186$) of the life shortening is due to excess mortality related to the occurrence of neoplastic disease. These observations quantitate our previous suggestion [12] that most or all of the excess risk at 6 R/day and below was due to tumor-related mortality. This is also consistent with other information from the study of irradiated animals and man [24,25].

Extrapolation to Man

There are no secret solutions to this problem. Any one of the mouse-based coefficients of injury regardless of dimensions can be translated to human equivalence. The underlying assumption is that the mortality rate slopes (Gompertz slopes) for mouse and man are in an inverse ratio of radiosensitivities. This is the approximately 30:1 mouse:man ratio noted earlier in this discussion. In addition, there is the assumption that the ratio of slopes, or the slope displacement, has an identical relationship to the reduction of life expectancy in both species, as postulated by Grahn [11]. A number of relationships between the Gompertz slope, the mortality ratio and the life expectancy have been and can be demonstrated. Among these, the ratios MAS_D/MAS_0 and k_D/k_0 are seen to be highly correlated, in terms of a power function, for all the separate strains and hybrids, which leads to our assumption that the slope displacement or mortality ratio is related to the fraction of life lost independently of species differences in life expectation.

In the present data, $\left[\frac{MAS_D}{MAS_0} \right] = \left[\frac{MR_D}{MR_0} \right]^{-.42} = (MR)^{-.42}$, since MR_0 always equals 1.0. The power function coefficient of -0.42 ± 0.01 is the common value among the four genetically different groups. Since the regression of $\ln MR$ on dose averages 0.00019 (Table III), that is, $MR_D/MR_0 = e^{0.00019D}$, it follows that the fraction of life lost per unit dose by the average mouse is equal to $1 - (MAS_D/MAS_0) = 1 - (MR)^{-.42} = 1 - (e^{0.00019D})^{-.42}$. The latter reduces to $(1 - e^{-8 \times 10^{-5}D})$ per R, or $(1 - e^{-9 \times 10^{-5}D})$ per rad. This is

approximately the same as the α term of $65 \times 10^{-6}/R$, given by Sacher [26] as the low dose-rate dose-effect constant for mice that was derived by a somewhat different approach [27,28].

A total dose of 250 rads delivered over the lifetime would produce 2.22% life shortening according to the data given here. This is 15 days for the average 100-day-old mouse and 445 days for the average 20-year-old human. Life shortening for man would amount to about 1.8 days per rad, which is slightly greater than the figure of 1.2 days derived on the basis of the relation between MAS and the daily exposure level. The comparison of these values does not involve a truly independent pair of estimates, however, because the same data contribute to both. Different analytical pathways are used, though they eventually converge on the assumption that man:mouse extrapolation relies upon the ratio of life table constants.

Finally, the above example suggests that a total lifetime dose equal to the present maximum permissible dose of 5 rem per year for 50 years of occupational exposure, delivered at the rate of about 100 mrem per week, would induce a 15-month reduction in life expectancy.

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LATE EFFECTS OF WHOLE- OR PARTIAL-BODY X-IRRADIATION ON MICE

Causes of death

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Abstract

LATE EFFECTS OF WHOLE- OR PARTIAL-BODY X-IRRADIATION ON MICE: CAUSES OF DEATH.

The primary aim of the present experiments was to compare the late effects of partial-body irradiation with those of whole-body irradiation. A total of 578 ddY female mice of 10 weeks old were assigned to the following five groups: (1) Whole-body exposure of 600 R, (2) head exposure of 800 R, (3) trunk exposure of 800 R, (4) lower body exposure of 800 R, (5) unirradiated control. Mean after-survival times of the above five groups were as follows with their standard errors: 69.2 ± 1.9 weeks for control, 43.0 ± 1.7 weeks for the whole-body exposure, 66.1 ± 2.2 weeks for the head exposure, 59.7 ± 1.6 weeks for the trunk exposure, 62.7 ± 1.8 weeks for the lower body exposure. The life-shortenings in the irradiated groups were statistically significant except for the head exposure. The life-shortenings expressed as a percentage of the life span in the control were: 6%/100 R with whole-body exposure, 2%/100 R with trunk exposure, and 1%/100 R with lower body exposure. The increases in incidence of all tumours and of malignant lymphomas were statistically significant in the group of whole-body exposure to 600 R. The head exposure to 800 R increases the incidence of pituitary tumours. In the trunk exposure, the incidence of ovarian tumours increased and malignant lymphomas decreased in incidence. The lower body exposure to 800 R did not change the spectrum of the incidences in the control. Calculating the mean after-survival time of mice who died from the same cause of death, reveals that most of the causes of death in the irradiated groups appeared earlier than in the control group. In particular, the mean after-survival time of the mice with malignant lymphoma in the whole-body exposure was 35 weeks, which was much earlier than the corresponding 73 weeks in the control. The increased life-shortening in the whole-body exposure was due to the high incidence of malignant lymphomas and to earlier appearance of malignant lymphomas, lung tumours and mammary tumours. The life-shortening by the partial-body exposure was much less than that by the whole-body exposure owing to the lack of induction of malignant lymphomas.

1. INTRODUCTION

It is generally accepted that a whole-body exposure to sublethal doses of ionizing radiation induces life shortening in animals [1, 2]. The life-shortening has also been observed after partial-body irradiation in mammals [3, 4]. As yet, however, the life-shortening and the cause of death from partial-body irradiation have not been systematically studied in any investigation. The present experiment was designed to study the long-term effects of partial-body exposure with special reference to life-shortening and tumour incidence. The data on life-shortening will be published elsewhere; the present report summarizes the pathological aspects of the study.

2. MATERIALS AND METHODS

The animals used throughout this work were ddY/SLC (closed colony) female mice of 10 weeks old. The X-ray apparatus was operated at 200 kVp, 20 mA, with filters of 0.5 mm Cu and 0.5 mm Al. Dosimetry was carried out with a Radocon Model 575. The range of the dose rate was 54 to 60 R/min. For partial body irradiation, the following three groups were assigned:

- (1) Heat exposure.
- (2) Trunk exposure (front legs, chest and abdomen).
- (3) Lower body exposure (pelvis, hind legs and tail).

The shield was of lead, 5 mm thick. The absorbed dose in the shielded area was a few per cent of that in the irradiated area. All the mice were anaesthetized by intraperitoneal injection of sodium 5-ethyl-5 (1-methylbutyl) barbiturate (0.1 mg/g body weight) during the irradiation. Before and after the irradiation, the mice were kept in rooms equipped with a forced-air ventilation system. The temperature of the rooms was $24^{\circ} \pm 1^{\circ}\text{C}$. Cage groups were not disturbed until the death of the last mouse. The mice were fed pelleted mouse food (MB-1, Funabashi Farm Co. Ltd) and tap water ad libitum. They were then checked daily for mortality throughout life and were necropsied on death. At necropsy all the animals were examined both grossly and microscopically. The tissues removed were processed in the usual manner and stained with haematoxylin and eosin.

TABLE I. MEAN AFTER-SURVIVAL TIMES (MAS) OF WHOLE- OR PARTIAL BODY X-IRRADIATION IN ddY FEMALE MICE

Area exposed	Dose (R)	No. of mice	MAS \pm S.E. (weeks)
None	0	116	69.2 \pm 1.9
Whole body	600	115	43.0 \pm 1.7**
Head	800	116	66.1 \pm 2.2
Trunk	800	114	57.9 \pm 1.6**
Lower body	800	117	62.7 \pm 1.8*

Statistically significant life shortening

** : $p < 0.01$, * : $0.01 < p < 0.05$.

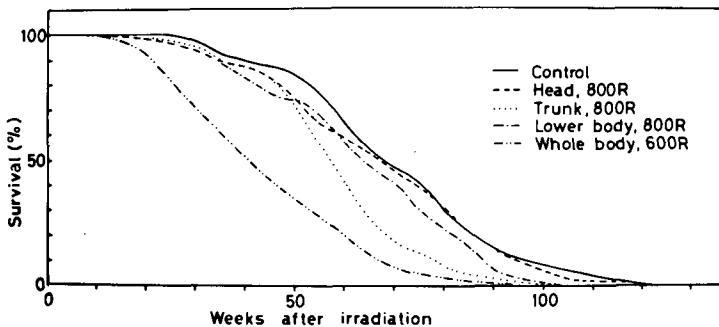


FIG.1. Survival curves of ddY female mice after whole- or partial-body irradiation. The irradiation was carried out at 10 weeks of age.

3. RESULTS

3.1. Life span data

The number of mice and X-ray doses administered to them are listed in Table I. All the X-ray doses were given as a single dose at 10 weeks of age. No death was observed within 30 days after the exposures apart from 4 mice in the whole-body exposure. These four mice are not included in Table I. A comparison of the mean after-survival times of the irradiated groups with that of non-irradiated, reveals statistically significant life shortenings in the groups of whole-body, trunk,

TABLE II. PERCENTAGE INCIDENCE OF CAUSES OF DEATH IN ddY FEMALE MICE

	Control	600R Whole body	800R Head	800R Trunk	800R Lower body
1. Neoplastic diseases	55.2	67.8 +	62.1	57.9	57.3
(a) Malignant lymphoma	20.7	36.5 *	15.5	7.9 **	14.5
(b) Pulmonary tumour	17.2	9.6	23.3	17.6	21.4
(c) Mammary tumour	12.1	10.4	9.5	9.6	12.0
(d) Ovarian tumour	1.7	4.3	2.6	13.2 **	0.9
(e) Other tumours	3.5	7.0	11.2 *	9.6	8.5
2. Inflammatory diseases	32.8	27.8	31.9	31.6	34.2
3. Others	10.3	3.5	5.2	7.0	6.8
4. Unknown	1.7	0.9	0.8	3.5	1.7
Total (%)	100.0	100.0	100.0	100.0	100.0
No. of mice	116	115	116	114	117

Statistical significance in comparison with the control incidence

+: $0.05 < p < 0.1$, *: $0.01 < p < 0.05$, **: $p < 0.01$.

TABLE III. NUMBER OF MICE WITH MALIGNANT LYMPHOMAS AND REGIONS OF THEIR OCCURRENCE

	Mediastinum	Abdomen
Control	6/116	17/116
Whole body 600 R	18/115	19/115
Trunk 800 R	5/114	2/114

and lower-body exposure as shown in Table I. If one expresses these life shortenings as a percentage of the mean after-survival time of the control group, one can obtain the following results: 6%/100 R in the whole-body exposure, 2%/100 R in the trunk exposure, and 1%/100 R in the lower-body exposure. Life shortening observed in the head exposure was not statistically significant. The survival curves of these mice are given in Fig. 1.

3.2. Causes of death

3.2.1. Incidences of causes of death

The diseases associated with deaths were classified into four categories: neoplastic diseases, inflammatory diseases, others and unknown. Neoplastic diseases were subdivided into malignant lymphoma, pulmonary tumour, mammary tumour, ovarian tumour and other tumours. The 'other tumours' include hepatoma, sarcomata other than malignant lymphoma, pituitary tumour, etc. The inflammatory diseases are purulent inflammation and abscess. 'Others' cover chronic endocarditis, chronic nephrosis, amyloidosis, etc. Table II summarizes the percentage incidence of each cause in each group. The number of mice in each group is equal to the number in Table I. Neoplastic diseases as a whole in the whole-body exposure increased (level of significance, $P < 0.1$), with the increase concentrated on malignant lymphoma ($p < 0.05$). The mice with malignant lymphoma in the control, in the whole-body exposure and in the trunk exposure were classified according to regions where the lymphoma was found. The results are shown in Table III; mice with leukaemia or with two lymphomas in both mediastinum and abdomen are excluded. It is clear that the whole-body exposure of 600 R induced specifically the malignant lymphoma in the mediastinum.

TABLE IV. MEAN AFTER-SURVIVAL TIMES OF EACH CAUSE OF DEATH IN WEEKS WITH STANDARD ERROR

	Control	600R Whole body	800R Head	800R Trunk	800R Lower body
1. Neoplastic diseases	72.4 ± 2.6	42.7 ± 2.2 **	73.0 ± 2.6	59.6 ± 2.0 **	63.4 ± 2.4 *
(a) Malignant lymphoma	73.1 ± 3.9	34.7 ± 2.3 **	71.6 ± 5.5	40.5 ± 5.8 **	59.1 ± 4.9 *
(b) Pulmonary tumour	75.5 ± 3.7	47.3 ± 5.2 **	75.2 ± 3.7	64.1 ± 3.9 *	57.2 ± 4.1 **
(c) Mammary tumour	62.7 ± 5.7	39.2 ± 4.0 **	59.0 ± 5.3	61.4 ± 3.2	70.0 ± 4.4
(d) Ovarian tumour	57.5 ± 11.0	64.1 ± 5.1	102.5 ± 6.5	68.4 ± 2.3	83.5
(e) Other tumours	93.5 ± 17.9	69.6 ± 6.2	75.3 ± 6.6	53.3 ± 3.1	74.8 ± 6.1
2. Inflammatory diseases	70.2 ± 3.1	45.3 ± 3.0 **	52.3 ± 3.5 **	55.3 ± 3.2 **	62.4 ± 3.1
3. Others	51.8 ± 5.5	31.0 ± 3.5 **	72.5 ± 10.2	53.5 ± 4.9	57.3 ± 8.9
4. Unknown	55.5 ± 2.0	57.5	47.5	61.0 ± 10.6	66.0 ± 8.5
All causes of death	69.2 ± 1.9	43.0 ± 1.7 **	66.1 ± 2.2	57.9 ± 1.6 **	62.7 ± 1.8 *

Statistical significance in comparison with mean after-survival time of the same cause of death in control

*: $0.01 < p < 0.05$, **: $p < 0.01$.

The exposure of the head to 800 R induced tumours classified as 'other tumours'. The tumours that increased were hypophysial adenoma, from 0/116 in the control to 5/116 in the head exposure ($p < 0.1$). In the case of trunk exposure, the incidence of malignant lymphomas decreased ($p < 0.01$). The malignant lymphomas of which the incidence was reduced by the trunk exposure were found in the abdomen, most of them in the mesenteric lymph nodes, and in the spleen, as shown in Table III. The other change of statistical significance in the trunk exposure is the increase in ovarian tumours which are rare in the control. In regard to the lower body exposure, none of the causes of death showed a difference with statistical significance compared with those in the control. From these facts, we conclude that the whole-body exposure increased malignant lymphomas, the head exposure increased pituitary tumours, and the trunk exposure induced an increase in ovarian tumours and decreased malignant lymphomas.

3.2.2. Mean after-survival times of causes of death

Table IV shows mean after-survival times of each cause for each group. Mean after-survival times of the neoplastic diseases as a whole were shortened in the irradiated groups with the exception of the head exposure. Age distribution of death from all neoplastic diseases is shown in Fig. 2. The distribution of the whole-body exposure was remarkably shifted to an earlier age. Three common tumours in the control, namely malignant lymphomas, pulmonary tumours and mammary tumours, were found at 62.7 weeks to 75.5 weeks after the beginning of observation. The exposure of the whole body to 600 R brought forward the time of deaths in the three tumours named above. In particular the change in malignant lymphomas is remarkably large where mean after-survival time of the whole-body exposure is 34.7 weeks in contrast to 73.1 weeks in the control. The age distribution of malignant lymphomas is shown in Fig. 3. It is clear that the peak of all tumours at 30 weeks of the whole-body exposure in Fig. 2 was primarily brought about by malignant lymphomas in the same group shown in Fig. 3. The acceleration in occurrence of the mammary tumours was observed only in the whole-body exposure. The age distribution of mammary tumours is given in Fig. 4. The large life-shortening with whole-body exposure seems to be due to the induction of malignant lymphomas and to the earlier onset of the tumours and of the inflammatory diseases.

In the head exposure the only change with statistical significance was a shortening of mean after-survival time of the inflammatory diseases, as shown in Table IV. The reason for the absence of shortening of the mean after-survival times of tumours may be in the fact that the tissues associated with the tumours were not irradiated in the head exposure, with the exception of the pituitary. Mean after-survival time of mice with pituitary tumour was 64 weeks in the head exposure, which was comparable with 66 weeks of the mean after-survival time from

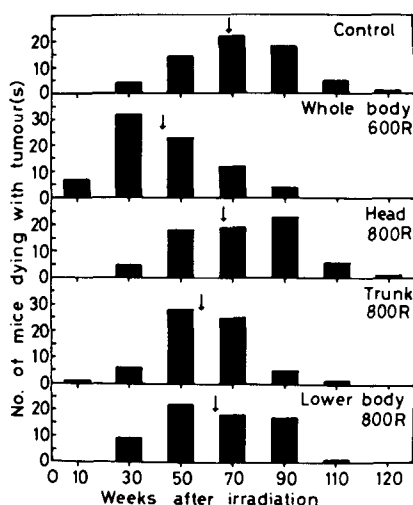


FIG.2. Age distribution of death from all neoplastic diseases in *ddY* female mice. Arrows indicate mean after-survival time from all causes in each group.

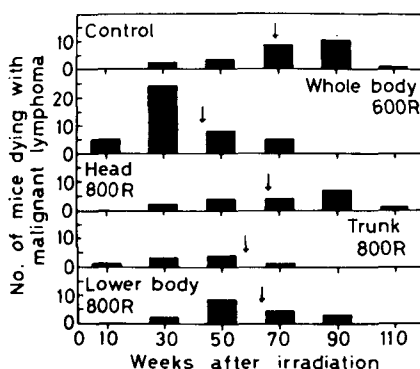


FIG.3. Age distribution of death with malignant lymphoma in *ddY* female mice. Arrows indicate mean after-survival time from all causes in each group.

all causes of death in the same group. A contribution by the induced pituitary tumours to life-shortening may not be large in the head exposure, the reasons being the small incidence and relatively late occurrence of these tumours. Although the trunk exposure induced ovarian tumours, their mean after-survival time was not shortened compared with that in the control. The induction of ovarian tumours therefore may not contribute markedly to the life-shortening in the trunk exposure. Mean after-survival times of the neoplastic diseases as a whole, malignant lymphomas and pulmonary tumours were reduced by the lower

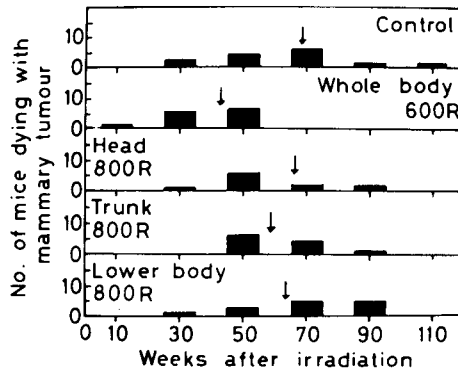


FIG.4. Age distribution of death with mammary tumour in ddY female mice. Arrows indicate mean after-survival time from all causes in each group.

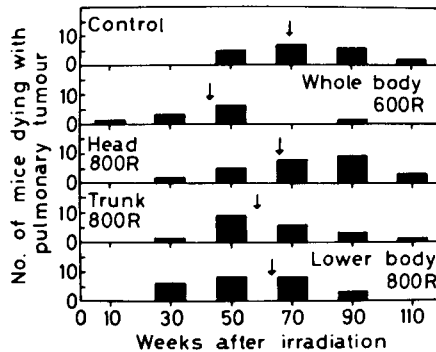


FIG.5. Age distribution of death with pulmonary tumour in ddY female mice. Arrows indicate mean after-survival time from all causes in each group.

body exposure (see Table IV and Fig. 5). Most of the malignant lymphomas in the lower body exposure were observed in the mediastinum and abdomen. There seems to be an abscopal effect on the time of occurrence of malignant lymphomas and pulmonary tumours from the irradiated lower body. Age distribution of inflammatory diseases is shown in Fig. 6 where the mean after-survival time was not shortened in the lower body exposure as an exception (see Table IV).

From the data mentioned above, we may conclude that the extensive life-shortening with whole-body exposure is primarily due to the induction of malignant lymphomas and to the earlier occurrence of diseases. In the partial body exposure, the earlier occurrence of diseases causes the life shortening, the extent of which is less owing to the lack of induction of malignant lymphoma.

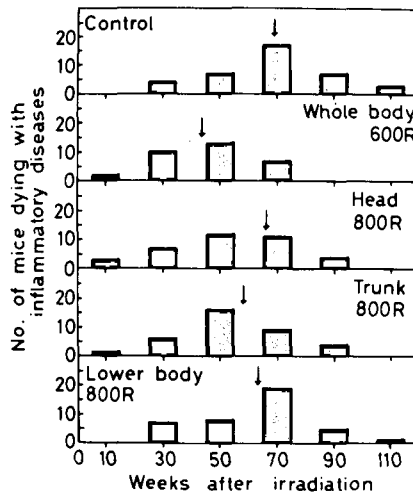


FIG. 6. Age distribution of death with inflammatory diseases in *ddY* female mice. Arrows indicate mean after-survival time from all causes in each group.

4. DISCUSSION

Thymic lymphomas induced by whole-body radiation were commonly observed in various strains of mice, namely C57BL [5], RFM [6], CF₁ [7], SAS [1], BC3F₁ [8], LAF₁ [9, 10], etc. The induced malignant lymphomas in the mediastinum presented in the text were not specified as thymic lymphomas partly because of the large mass of the tumour and autolysis of the specimens. A protection against the development of radiation-induced thymic lymphoma by partial body irradiation of mice was found by many investigators [5, 11, 12]. Although the partial body exposure adopted in the present study also inhibited the induction of malignant lymphomas in the mediastinum, the trunk exposure decreased the incidence of abdominal lymphomas below that in the control. The whole-body exposure generally reduces non-thymic lymphomas [9, 10, 13] in mice, and its implication may be that the exposure reduces the population of mice at risk through mortality from earlier radiation-induced lesions [13]. The decrease of abdominal lymphomas by the trunk exposure may not be related to the earlier occurrence of thymic lymphomas as a competing risk since only 5 mice out of 114 mice in this case died with lymphoma, as shown in Table III. The ovarian tumours induced by the trunk exposure cannot be a competing risk against the abdominal lymphoma since the ovarian tumours occurred late in life, as seen in Table IV. Another explanation will therefore be required for the decrease of abdominal lymphomas by the trunk exposure. Chest exposure of 750 R of X-rays induced pulmonary tumours with a high incidence in RFM male

mice [14]. The trunk exposure of 800 R induced no more lung tumours than in the control in ddY/SLC female mice, as shown in Table II, and the difference between data on RFM and ddY/SLC may be brought about by the differences in the irradiated area and in the technique to detect the lung tumours, among other factors, e.g. strains, sexes and observation periods. Hirose and co-workers have recently induced rectal carcinomas by irradiating the pelvic region of ICR and CF₁ mice with 3000 R of X-rays [15]. The dose of 800 R to the trunk may be too low to induce a rectal carcinoma. Ovarian tumours in mice are in contrast to the mammary tumours in the sense that radiation easily induces ovarian tumours [1, 6, 8–10, 13, 16]. The induction of ovarian tumours by the trunk exposure might compete with the induction of the lung tumours, as suggested in Tables II and IV. Komuro has found ovarian tumours with high incidence in ddY/F with 260 R of X-rays to the whole body [17]. The higher incidence in the ddY/F mice may be brought about by the younger age at exposure [12, 18]. Pituitary tumours reported by Yokoro and co-workers [19] were induced by head exposure of 775 R of X-rays on LAF₁ female mice. Their incidence of 4.8% was close to 4.3% (5/116) from the head exposure of 800 R to ddY/SLC mice in the present report. On the other hand, Ullrich and co-workers have reported 21% of pituitary tumours on RFMf/Un females, SPF, by 300 rads of gamma rays to the whole body [6].

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DISCUSSION

D.J. MEWISSEN: Your data show that the observed incidence of lung tumours is quite variable, depending on the site of partial irradiation. The highest incidence arose following cephalic irradiation, that is, excluding the thorax. How do you interpret these findings?

F. SATO: The incidence of pulmonary tumours in mice generally decreases with doses to their whole bodies. The reason is that many mice die earlier with malignant lymphoma and only a small number of mice live long enough to develop pulmonary tumours. The appearance times of pulmonary tumours are not accelerated much by radiation. This is a problem of competing risks — between malignant lymphoma and lung tumour. Accordingly, in the case of substantial life-shortening one will not expect pulmonary tumours. Life-shortening is least with irradiation of the head. This may be an explanation for the high incidence of pulmonary tumours associated with irradiation of the head.

D. GRAHN: A correction for competing risks must be made. In the paper I have presented¹ one method is given, and that is to use age-standardized mortality ratios or age-standardized measures of the number of excess deaths. If this is done, then, for example, the excess risk of pulmonary tumour mortality rises steadily with increasing total dose within and across dose rates up to 12 R/d (10.8 rads/d), even though percentage incidences drop steadily. This type of correction is especially important when analysing tumour data over a wide dose range and for tumours that occur late in life.

F. SATO: I agree with you. The trouble is that so many methods have been proposed up to now and the corrected incidence may depend upon the method used.

R.L. ULLRICH (*Chairman*): I should also like to emphasize the importance of correcting for competing risks in experimental studies. Interpretation of studies based on observed incidences is very difficult. With respect to this, do you think that the different incidences of ovarian tumours after whole-body and trunk irradiation may be due to the fact that whole-body irradiated animals develop malignant lymphoma and trunk-irradiated animals do not?

F. SATO: Yes, I think so.

¹ GRAHN, D., SACHER, G.A., LEA, R.A., FRY, R.J.M., RUST, J.H., "Analytical approaches to and interpretations of data on time, rate and cause of death of mice exposed to external gamma irradiation", these Proceedings 2, IAEA-SM-224/209.

RELATIONSHIP OF DOSE RATE AND TOTAL DOSE TO RESPONSES OF CONTINUOUSLY IRRADIATED BEAGLES*

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Abstract

RELATIONSHIP OF DOSE RATE AND TOTAL DOSE TO RESPONSES OF CONTINUOUSLY IRRADIATED BEAGLES.

Young-adult beagles were exposed continuously (22 hours/day) to ^{60}Co gamma rays in a specially constructed facility. The exposure rates were 5, 10, 17 or 35 R/day, and the exposures were terminated at 600, 1400, 2000 or 4000 R. A total of 354 dogs were irradiated; 221 are still alive as long-term survivors, some after more than 2000 days. The data on survival of these dogs, coupled with data from similar preliminary experiments, allow an estimate of the LD_{50} for gamma-ray exposures given at a number of exposure rates. They also allow comparison of the relative importance of dose rate and total dose, and the interaction of these two variables, in the early and late effects after protracted irradiation. The LD_{50} for the beagle increases from 344 R (258 rads) delivered at 15 R/minute to ~ 4000 R (~ 3000 rads) at 10 R/day. Over this entire range, the LD_{50} is dependent upon haematopoietic damage. At 5 R/day and less, no definitive LD_{50} can be determined; there is nearly normal continued haematopoietic function, survival is prolonged, and the dogs manifest varied individual responses in other organ systems. Although the experiment is not complete, interim data allow several important conclusions. Terminated exposures, while not as effective as irradiation continued until death, can produce myelogenous leukaemia at the same exposure rate, 10 R/day. More importantly, at the same total accumulated dose, lower exposure rates appear more damaging than higher rates on the basis of the rate and degree of haematological recovery that occurs after termination of irradiation. Thus, the rate of haematologic depression, the nadir of the depression and the rate of recovery are dependent upon exposure rate; the latter is inversely related and the first two are directly related to exposure rate.

INTRODUCTION

Studies of protracted irradiation have largely focused on the results of irradiation given until death, and many have used intermittent, discontinuous exposures [1-7]. Mammals given whole-body irradiation from high

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energy γ -ray sources develop symptoms and lesions and have associated survival times that are highly dependent upon both total dose and dose rate [1-8]. Exposure rates in excess of 5 R/minute produce symptomatology that is determined primarily by total dose. At these high exposure rates, a few hundred rads is a midlethal or LD₅₀ dose and death results within a few days to a few weeks [9]. Changes in total dose of a few rads, more or less, determine the death or recovery of the animal. Our own studies with dogs irradiated continuously until death have shown that exposure rates above 5 R/day result in deaths that are related solely to damage to the hematopoietic system [10-12]. Although the immediate causes of death vary, the basic damage is to the orderly production of the cellular elements of the blood. Differential damage to production and maturation of granulocytes and erythrocytes leads to death from either septicemia or anemia. At exposure rates of 5-10 R/day, there is early suppression of peripheral blood values, but in ~50% of exposed animals there is recovery toward normal values indicating an accommodation to the radiotoxic affects. This recovery or accommodation phenomenon leads eventually to hyperplasia of the granulocytes and ultimately to death from leukemia. This syndrome, hematopoietic perturbation leading to leukemia, will be discussed in another paper at this symposium [13].

When whole-body irradiation is continued until death, the causes of death are highly correlated with dose rate, and bone marrow is the critical tissue. However, when the irradiation is terminated at predetermined levels of total exposure, the results are quite different. The animals can recover from or escape the acute hematological effects, and the relative importance of dose and dose rate is less well known [7,13,14]. Further, although the life shortening aspects of continuous irradiation (time to death and dose rate effects) have been evaluated at this Laboratory and by other workers, the physiological, clinical, organ, and tissue responses have received less critical evaluation, particularly in long-lived animals [1,15,16].

This report summarizes interim data from an ongoing study of the late effects of predetermined total exposures of whole-body ⁶⁰Co γ irradiation given to beagle dogs at several daily exposure rates. The objective is to determine whether or not the late effects produced by a given total dose of irradiation are significantly influenced by dose rate.

MATERIALS AND METHODS

A description of the closed beagle colony at Argonne and its management has been published previously [17].

Groups of young-adult beagles of both sexes (~400 day old) were exposed 22 hours/day at four different exposure rates to predetermined total exposures of ⁶⁰Co γ -rays in a specially constructed facility [8]. Total exposures of 600 and 1400 R were given at 35, 17, 10, and 5 R/day; 2000 R was given at 17, 10, or 5 R/day; 4000 R at 10 and 17 R/day. Enough dogs were irradiated in each of the above groups to produce approximately 20 dogs that survived more than 100 days after the termination of irradiation. High mortality at total exposures above 2000 and 1400 R given at 17 and 35 R/day, respectively, and the extremely long times required for an exposure of 4000 R at 5 R/day limited the exposure groups.

Each dog was evaluated regularly by clinical examinations, hematology, and blood chemistry. All dogs becoming moribund or dying were necropsied and tissues were fixed, sectioned, and examined by light and electron microscopy.

RESULTS AND DISCUSSION

A total of 354 dogs were irradiated in 13 groups. As shown in Table I, a total of 221 are still alive (January 1, 1978). There were 58 decedents during irradiation, 44 during the first 100 days following termination of irradiation, and 31 to date at times greater than 100 days following termination of irradiation.

It is apparent from the clinical, hematological, and pathological data that damage to the hematopoietic system resulting in septicemia or anemia is the sole cause of death of dogs that died during irradiation, and for the first 100 days following irradiation. Among the 31 dogs dying at times greater than 100 days after completion of irradiation, hematopoietic damage or dysfunction resulted in the death of only 10 dogs: 2 cases of anemia due to marrow aplasia; 5 cases of myeloproliferative disorder (MPD) manifested as myelogenous leukemia or monocytic leukemia; and 3 malignant lymphomas (lymphosarcomas). The nature of the hematopoietic dysfunctions was significantly altered as compared to those in early deaths, at each of the four exposure rates; there was hyperfunction (neoplasia) resulting in leukemias or related disorders in 8, and hypofunction (anemia) in only 2.

Nine nonhematopoietic malignancies also occurred among the 31 decedents. These included a carcinoma of the intestine, a squamous carcinoma of the buccal cavity, 2 angiosarcomas of the spleen, a generalized cutaneous mast cell tumor, 2 mammary carcinomas, 1 splenic neurofibrosarcoma, and 1 transitional cell tumor of the bladder.

It is premature to predict the frequency of malignancies that will occur among the remaining dogs, but the data to date can be compared to that in other irradiated and untreated dogs in our colony. A survey of tumors among untreated control and breeder animals in the colony [18] allows us to conclude that although the incidence of spontaneous tumors is high among aged control animals, the tumor types, numbers, and ages at the time of death from malignancies already seen in this study are significant. The occurrence of MPD's are particularly noteworthy as none has been seen in our untreated dogs.

Among dogs irradiated at 5 and 10 R/day until death in our earlier studies, the incidence of MPD was ~50% [8,10-13]. At 5 R/day, deaths from MPD ranged from 989 to 1949 days after beginning of irradiation, while at 10 R/day MPD deaths occurred between 383 and 1622 days of irradiation. In the terminated exposures being considered here, the three MPD deaths in the 10 R/day group (4000 R total exposure) were at 655 to 981 days following beginning of irradiation. The other two cases of MPD were in the groups irradiated at 5 and 17 R/day for total exposures of 2000 R and occurred at 729 and 1358 days, respectively, after beginning of irradiation. In each of the three irradiation groups where MPD has occurred, the decedents with MPD were, with one exception, the first to die in the period more than 100 days after irradiation. All earlier

TABLE I. EFFECT OF CONTINUOUS IRRADIATION

Exp. Rate	Total Exp.	Total Dogs	Number Dead and Cause ^a				Number Alive and Age (in days) ^b	
			During Irradiation	After Irradiation				
				< 100 days	> 100 days	No. days ^a		
5 R	600 R	20	0	0	0		20 438-489	
	1400 R	24	0	0	1-lymphoma	(931)	23 1400-2106	
	2000 R	20	0	0	1-MPD	(729)	19 993-1393	
10 R	600 R	20	0	0	0		20 309-663	
	1400 R	24	2-S	2-A	1-paresis & hydrocephalus		18 1233-1380	
					1-heartworms			
	2000 R	28	2-A	1-S	1-anemia		16 1653-1898	
				6-A	1-pneumonia			
					1-epilepsy			
	4000 R	31	1-S	3-A	3-MPD	(655,812,981)	8 2101-2794	
			8-A		1-lymphoma	(1016)		
					2-pneumonia			
					1-intestinal carcinoma	(2375)		
17 R	600 R	20	0	0	0		20 257-660	
	1400 R	25	0	3-A	0		22 884-1066	
	2000 R	53	17-S	6-A	1-anemia		18 102-1843	
			3-A	6-S	1-MPD (monocytic leukemia)	(1358)		
				1-H				
	4000 R	24	6-A	1-A	2-pneumonia		8 2347-2893	
			7-S					
	35 R	600 R	20	0	0	0	20 262-438	
	1400 R	45	12-S	15-S	3-endometritis (& 1 bladder tumor)	(2889)	9 2893-3411	
					1-enteritis			
				1-splenic hematoma				
				1-lymphoma	(2381)			
				1-neurofibrosarcoma	(2516)			
				1-mast cell tumor	(2899)			
				1-angiosarcoma	(775)			
		354	58	44	31		221	

^aS = septicemia; A = anemia, MPD = myeloproliferative disorder; H = hemorrhage.

^bNo. days since beginning of irradiation. Because dogs were not all irradiated at one time the time of survival varies within each group.

deaths were acute deaths from anemia or septicemia as the result of bone marrow suppression. There were similar results in 8 other dogs given terminated exposures of 1700 R at 17 R/day in a preliminary study. Three died of anemia and septicemia less than 200 days after beginning of irradiation, two died of leukemia at 400-500 days and the remainder at later times of other causes [19]. MPD also occurred in dogs irradiated continuously until death in our previous study as a predictable sequel following a nadir and recovery of blood values [13].

It is unlikely that any more of the dogs given terminated exposures will die with MPD; many have already survived longer following irradiation than those that developed MPD, and none have yet developed clinical or hematological signs of the disease.

No dog given terminated exposures at a rate of 35 R/day has developed myelogenous leukemia. Because irradiation given at 35 R/day causes severe hematopoietic depression in dogs given a total exposure of 1400 R ($\sim 60\%$ die within 100 days) and rapid recovery is possible, it must be concluded that either the dose rate or total dose are ineffective for leukemia induction. At this time neither of the alternatives can be eliminated or proven, and it may be that both are correct.

Among dogs irradiated until death, the cases of MPD occurred as a cluster or wave of deaths as did anemias and septicemias [8,12]. When dogs were left in the irradiation field at 10 R/day until they died, MPD was the cause of death in that 50% of the group surviving longest. At 5 R/day, however, MPD occurred in the 50% dying earliest, and a variety of other malignancies, degenerative and inflammatory processes occurred later [8,10]. Taken as a whole, these data on MPD, from lifetime and terminated exposures, suggest that there are relationships between dose and dose rate for induction of leukemia. It is obvious that there are responses of the marrow that are a prerequisite to leukemia [13].

Two of the three lymphomas in the present study also occurred early among the chronic survivors. One case caused the first chronic death in the 5 R/day, 1400 R total exposure group, and the other was the cause of death in the fourth decedent at 10 R/day, 4000 R total exposure, following the first three deaths due to MPD. The remaining lymphoma occurred after more than 2300 days in the 35 R/day, 1400 total dose group.

Although the data from these terminated exposures are directed toward the study of late effects, when tabulated in conjunction with other previously published data, they allow for estimations of the LD_{50} for deaths (hematopoietic damage) associated with varying exposure rates. As shown in Table II, a reduction in the exposure rate increases the amount of radiation required to kill (LD_{50}), a concept that is well established but for which data at low dose rates in larger, longer-lived animals are difficult to obtain [1]. The data presented here also show that as the exposure rate is decreased, the ability to measure or determine an LD_{50} dose becomes increasingly difficult, if not impossible. At 5 R/day (Table I) there were no acute hematopoietic deaths in any of the groups, including that given the highest total exposure of 2000 R. Similarly, even when irradiation was continued until death at 5 R/day in our earlier study, there were only four deaths due to hematopoietic suppression and the earliest of these was at 390 days of irradiation [8,10]. It is clear that as the exposure rate is decreased, the percentage of dogs surviving to show late effects increases, and that below 10 R/day the most sensitive tissue or

TABLE II. EFFECT OF EXPOSURE RATE OF TERMINATED EXPOSURES OF ⁶⁰Co γ-RAYS ON SURVIVAL OF BEAGLES

Exposure Rate (Roentgens)	LD ₅₀ Estimate for Hematopoietic Damage (Average Absorbed Dose, rads) ^a	Reference
15 R/minute (21,600 R/day)	= 258 (mean time of death = ~ 20 days)	17
.0424 R/minute (61 R/day)	= ~ 500 { (4/4 died in mean of 21 days after 594 rads) (0/4 died after 412 rads)	8 8
.0243 R/minute (35 R/day)	= ~ 1050 (27/45 died in mean of 41.8 days after 1050 rads)	Table I
.0170 R/minute (24.5 R/day)	= ~ 1950 (3/8 died in mean of 113 days after 1837 rads)	19
.0118 R/minute (17 R/day)	= ~ 1500 (33/53 died in mean of 121.8 days after 1500 rads)	Table I
.0069 R/minute (10 R/day)	= 2250-3000 { (9/28 died in mean of 214 days after 1500 rads) (12/31 died in mean of 291.6 days after 3000 rads)	Table I
.0034 R/minute (5 R/day)	= ~ 7000 (12/24 died in 1865 days with continuous irradiation)	8

^aThe rad dose is calculated as .75 x exposure in R (Reference 20).

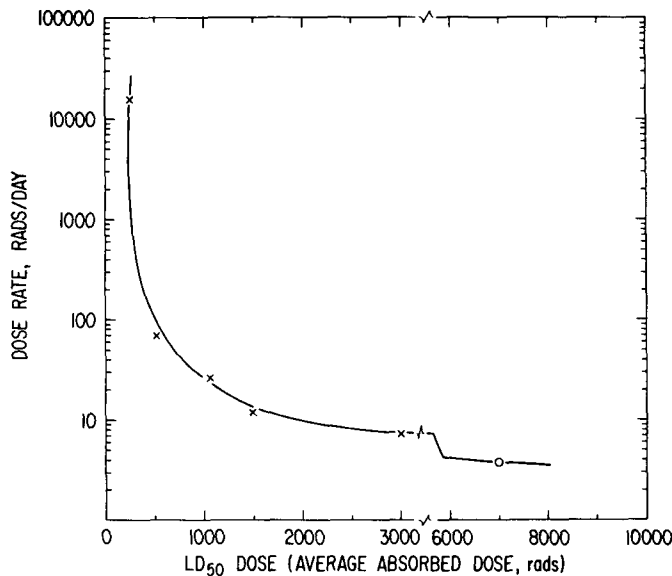


FIG.1. LD₅₀ dose for dogs given whole-body gamma irradiation at various dose rates. Sources of the data are given in Table II. The value at 3.75 rads/day is for continuous irradiation; all other values are for terminated exposures.

target organ shifts from the hematopoietic system to other organs or systems. This shift from acute hematopoietic damage and early death to longer survival times is seen when the data from Table II are plotted as in Fig. 1. Here the data are plotted as the average absorbed dose, in rads [20], to allow comparisons to other experiments and animal species. At the lower dose rates, small changes in dose rate produce large variations in the LD₅₀ dose. This effect is an expression of the increasing ability of the individual animal to accommodate to the irradiation damage and of variability in the site of damage, and therefore in the causes of death. Specifically, decreasing the dose rate from 26.25 to 7.5 rad/day (35 to 10 R/day) increases the LD₅₀ dose from 1050 to at least 3000 rad while increasing the dose rate from 26.25 to over 16,000 rad/day (35 and 21,600 R, respectively) only decreases the LD₅₀ from 1050 to 258 rad.

The hematologic response as measured by peripheral blood values is shown in Figures 2-4 where the data are plotted for dogs irradiated at 5, 10, 17, and 35 R/day to a total exposure of 1400 R. Because size appreciably influences absorbed dose [19], five male dogs 10 000-12 000 grams in weight that survived more than 100 days after irradiation were selected to represent responses for each exposure rate group.

Although the changes in erythrocyte numbers (Fig. 2) were not as dramatic as those of the other cellular components, a depression and recovery associated with irradiation exposure and its termination was suggested even at the lowest dose rate (5 R/day). Oscillations in the values tended to obscure the responses but there are particularly clear changes in the three higher dose rates. At 35 R/day there was an abrupt depression and similarly abrupt recovery in the erythrocyte numbers over a period of approximately 50 days after termination of radiation. The apparent delay is undoubtedly related to the lag time for depression of bone marrow function to be expressed as lowered peripheral blood values, assuming an average life-span of 100 days for the dog erythrocyte. Somewhat less expected was an apparent overshoot of the recovery of the erythrocyte values within 100 days after irradiation. Although preirradiation mean values were highest for this group, they tend to show a higher overcompensation than other groups when irradiation was terminated.

The responses of erythrocytes in the dogs in the 17 R/day group were similar to those at 35 R/day except that depressed erythrocyte values occurred during irradiation because of the longer time (82 days vs. 40 for 35 R/day) of irradiation, and recovery to higher than normal concentrations occurred at a slightly slower rate.

At 10 and 5 R/day the depression and recovery are less clear, and the overshoot as seen at 17 and 35 R/day is equivocal without additional data and analyses.

Changes in leukocytes, as shown in Fig. 3, were much greater at the highest exposure rate, but it is clear, even from these preliminary data, that there was a significant depression at all four exposure rates. Also, the higher the exposure rate, the more rapid the recovery following termination of irradiation. The platelet responses (Fig. 4) resembled those in the leukocytes, but it must be remembered that the numbers plotted in the case of leukocytes represent a mixed population--granulocytes, lymphocytes and other cells. In the case of platelets, the population being monitored is homogeneous, and therefore the similarity between the platelet and leukocyte response includes the correlation of the nadir, rate of decline, and rate

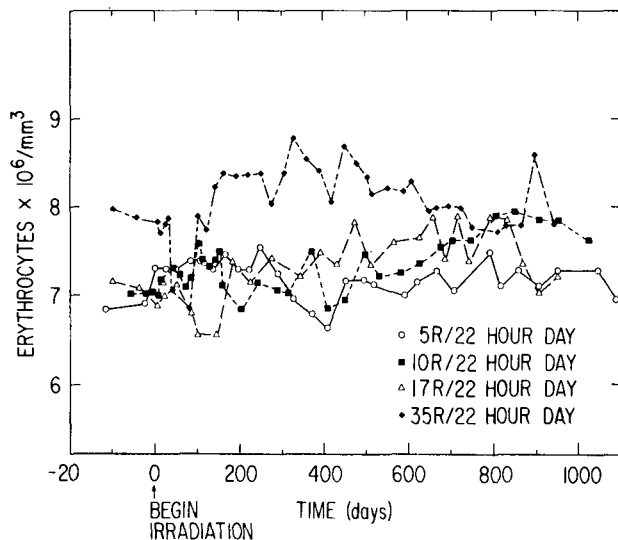


FIG.2. Erythrocyte values in dogs given 1400 R whole-body gamma irradiation at four exposure rates. Each point is the mean value derived from five dogs.

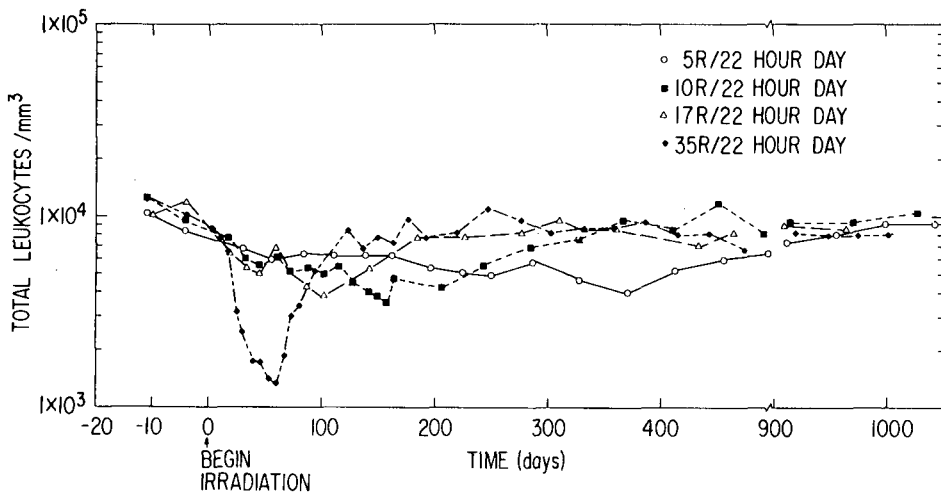


FIG.3. Total leukocyte values in dogs given 1400 R whole-body gamma irradiation at four exposure rates. Each point is the mean value derived from five dogs.

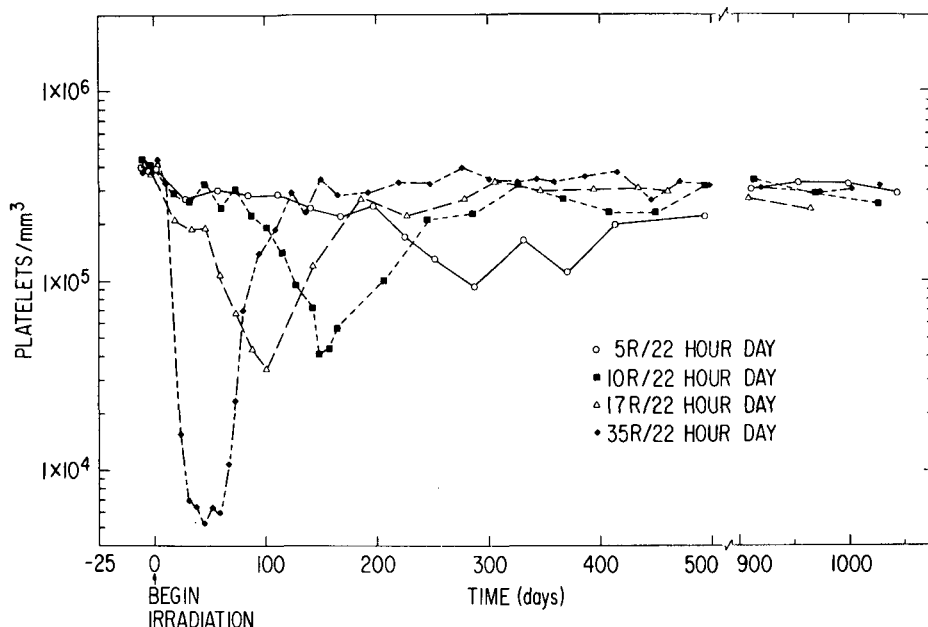


FIG.4. Platelet values in dogs given 1400 R whole-body gamma irradiation at four exposure rates. Each point is the mean value derived from five dogs.

of recovery. The nadir reached in the case of the groups at 10 and 17 R/day, and their respective decline and recovery, are reasonably similar, but obviously different from those at 35 and 5 R/day.

The fact that the rate of recovery of platelets and leukocytes is more rapid at the higher dose rates seems somewhat paradoxical and raises questions regarding the controlling mechanisms. Possible hypotheses, which are not necessarily mutually exclusive, include the levels of poietins or circulating humoral factors related to the severity of the depression of the peripheral blood cells, and changes in the kinetics of bone marrow cell differentiation and maturation. In previous studies of the hematopoietic effects of single, brief, near-lethal doses of irradiation, and irradiation given continuously to death, these dose rate related differences were not observed. Dogs surviving brief single near-lethal doses had depression and recovery rates related to the total dose [17]. Although the nadir and rate of depression of peripheral blood values were related to the dose rate in dogs irradiated until death (lower nadir and faster decline associated with higher dose rates), those dogs showing recovery in the face of continuing irradiation did so at essentially the same rate and independent of dose rate once the recovery pattern was established [8].

CONCLUSIONS

Although incomplete, the interim data from this study allow tentative conclusions. A most important conclusion is that myelogenous leukemia and related disorders occur in dogs given terminated exposures to continuous irradiation. Although not as effective as irradiation given until death ($\sim 50\%$ incidence in dogs given 5 and 10 R/day) at similar exposure rates, there have been 5 cases among 31 dogs surviving more than 100 days after termination of irradiation. The occurrence of three cases of malignant lymphomas also seems highly significant.

Myelogenous leukemia occurs at the same exposure rates as in dogs given irradiation until death. Furthermore, it occurs in association with recovery of the bone marrow after termination of irradiation and not at later times. This same sequence of events--marrow depression, recovery, and subsequent onset of leukemia--has been recorded by us in dogs irradiated continuously until death except that hematopoietic recovery occurs while irradiation continues.

The large number of other malignancies observed among the small number of decedents to date in the present study suggests that tumors of the soft tissues will be significantly increased compared to controls. No effect of dose or dose rate on induction of tumors other than myelogenous leukemia is apparent at this time.

Based on preliminary analysis of the response of the peripheral blood values, there is a direct correlation, not unexpected, between the rate and depth of depression of these values and the exposure rate when similar total exposures are given. Unexpected, however, is the more rapid recovery of these values, after termination of irradiation, in the dogs in the higher exposure rate groups. This inverse relationship between rate of recovery and exposure rate requires additional analyses for confirmation and explanation.

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DISCUSSION

R.L. ULLRICH (*Chairman*): Could you comment on why low dose rates are apparently more effective in inducing myeloproliferative disorders (MPD) than high dose rates?

T.E. FRITZ: At the higher exposure rates (greater than 17 R/day) there is suppression of the bone marrow, and anaemia or septicaemia result. Only at exposures of 5 and 10 R/day can the bone marrow continue to function for long periods of time. Eventually, with continuous exposure at these lower rates, there are changes in the marrow differentiation and maturation which result in hyperplasia and then leukaemia (MPD).

J.J. BROERSE: In a number of papers presented in this Session information has been given only on the exposure (in R) at the position of the biological object. I would strongly recommend that the absorbed dose (either in rads or in grays) be indicated, as this will make it easier to evaluate the biological results. From one of your slides I noticed that you used a factor of 0.75 for the R to rad conversion. I should be interested to know what the homogeneity of dose distribution over the dogs was. Did you make any correction for differences in weight of the animals, and did you take any steps to achieve bilateral irradiation of the dogs?

T.E. FRITZ: The factor of 0.75 for conversion of R to rad dosage is derived from the work of Sinclair (Ref.[20] of the paper). In order to achieve the most uniform dose distribution possible in our dogs, we have resorted to a system of rotating the exposure cages 90° each day. In this way the dogs are randomized with reference to their location in the cage and orientation to the ^{60}Co source. We have learned from observation of the dogs that they prefer to sit, lie or sleep at the back of the cage and away from the door. By rotating the cages each day we achieve exposures that approach bilateral exposures. With respect to body weight or size, we take these measurements into consideration when calculating rad dosages, as illustrated in one of our previous publications (Ref.[17] of the paper). All the haematological data presented in the paper are for dogs 10–12 kg in weight.

RELATIVE EFFECTS OF UNIFORM AND NON-UNIFORM EXTERNAL RADIATION ON THE INDUCTION OF LUNG TUMOURS IN MICE

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Abstract

RELATIVE EFFECTS OF UNIFORM AND NON-UNIFORM EXTERNAL RADIATION ON THE INDUCTION OF LUNG TUMOURS IN MICE.

Mice were irradiated with 200 kVp X-rays either uniformly to the whole lung or via 72 cylindrical 'microbeams' ~ 1 mm in diameter. The volume of tissue exposed via the 'microbeams' allowing for respiratory movements was $\sim 20\%$ of the total lung volume. The mice were sacrificed 12 months after irradiation, and the lungs inflated and cleared, which allowed detection of tumours down to ~ 0.5 mm diameter throughout the lung. Each geometry of irradiation produced a peaked dose-response curve for tumour induction. For uniform whole-lung irradiation the tumour incidence rose from a control value of 18% to a 47% at a dose of 5 Gy, and then dropped to control values at 7.5 and 10 Gy. The 'microbeam' irradiation gave a peak of 57% tumour incidence at 1 Gy, and then fell back to control values at 2.5 and 5 Gy with a slight increase at 7.5 Gy. In the 'microbeam' irradiation the peak dose of 1 Gy is the dose averaged over the whole lung, and so the actual dose received by the irradiated portion of the lung is 5 Gy. These results suggest that it may not be justifiable to use average tissue dose to make risk estimates for carcinogenicity. The important factor is the dose received by the tissue that is irradiated, and in this experiment the volume of tissue irradiated is immaterial. The results do not, however, give any indication as to the lower limit of volume of tissue irradiated to produce the same incidence.

INTRODUCTION

It is generally agreed that the induction of malignancies represents the most important hazard of radiation. In human exposures the radiation is rarely delivered uniformly, and epidemiological data are often derived from non-uniform or partial body exposures. It is important for radiation protection to design experimental studies of carcinogenic risks involving comparisons of uniform and non-uniform radiation exposures. This is of particular relevance in assessing tumour induction in the lung where radiation from inhaled alpha and beta emitters produces highly non-uniform spatial dose distributions.

At present there is no consensus as to the effect on tumour induction of varying dose distribution; for radiation protection purposes average tissue dose is considered to be appropriate for making risk estimates. This is based on the theory that cancer induction is stochastic, and so uniform irradiation of the whole organ or tissue will put the maximum number of cells at risk. Hence the risk estimate would be the most conservative in any exposure regardless of the uniformity of the dose. In addition to this it is believed that the high local doses associated with extremely inhomogeneous irradiation ('hot spots') may cause the death of potential cancer cells.

The concept of the importance of the size of the cell population at risk, implicitly accepted for many years, has recently been mathematically analysed in detail by Mayneord and Clarke (1). They conclude that when a tissue is irradiated the number of cells exposed profoundly influences the probability of tumour induction. There are some experimental data that support this idea that cancer production depends on the amount of tissue exposed. In studies of radiation-induced mammary tumours in rats, Bond, Shellabarger, Cronkite and Fliedner (2) exposed the animals to whole or partial-body irradiation and showed that the number of mammary cancers was directly proportional to the volume of tissue irradiated. Hulse and Mole (3) showed that the number of skin tumours induced in mice was approximately proportional to the area of skin irradiated.

The results given in this paper are the first from a series of experiments designed to test whether the critical factor for radiation carcinogenesis in the lung is the number of cells irradiated or whether it is the absorbed dose (almost irrespective of the fraction of cells absorbing it) that is the more critical.

MATERIALS AND METHODS

Groups of 3 month-old SAS/4 mice (16♂ and 16♀) were irradiated with X-rays either uniformly to the whole lung or with 72 cylindrical 'microbeams' 1 mm in diameter.

The radiations were carried out at a dose rate of 0.6 Gy/min using 200 kVp X-rays at 15 mA with 0.53 mm Sn, 0.25 mm Cu and 1.19 mm Al filters, HVL 2.6 mm Cu. The mice were shielded with 1 cm lead except for the thoracic region which was completely unshielded in the case of uniform irradiations, and shielded with 1 cm of lead drilled with 72 1 mm diameter holes for the 'microbeam' irradiations. The alignment of the X-ray beam with the holes and the scatter through the mouse were checked photographically using Kodak Fine Grain Safety Positive film. The dose pattern was maintained through the mouse and showed negligible scatter.

During irradiations dosimetry was carried out with a 0.2 cc Farmer Ionization Chamber in conjunction with thermoluminescent dosimetry using lithium fluoride sachets.

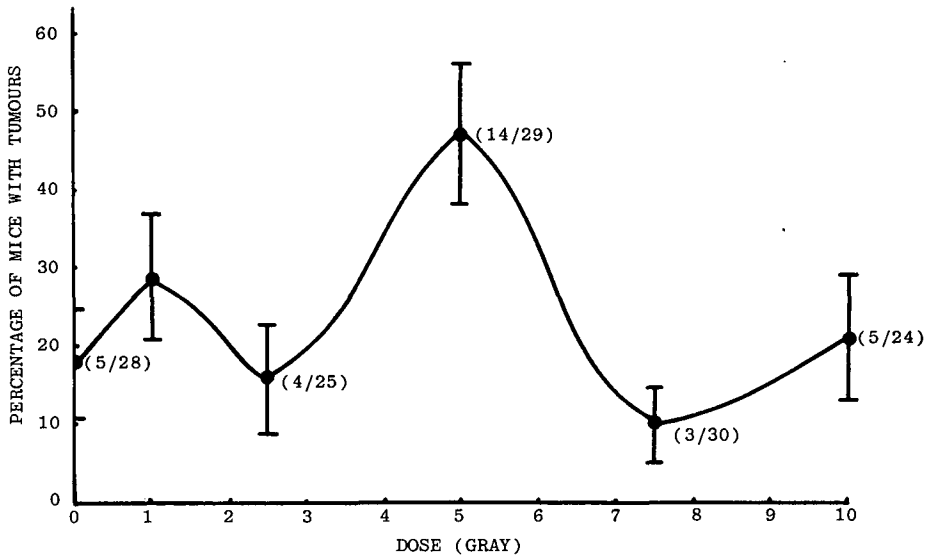


FIG.1. Percentage of mice with primary lung tumours (\pm S.E.) after uniform whole-lung irradiation. Figures in brackets indicate (number of mice with tumours)/(total number of mice in group).

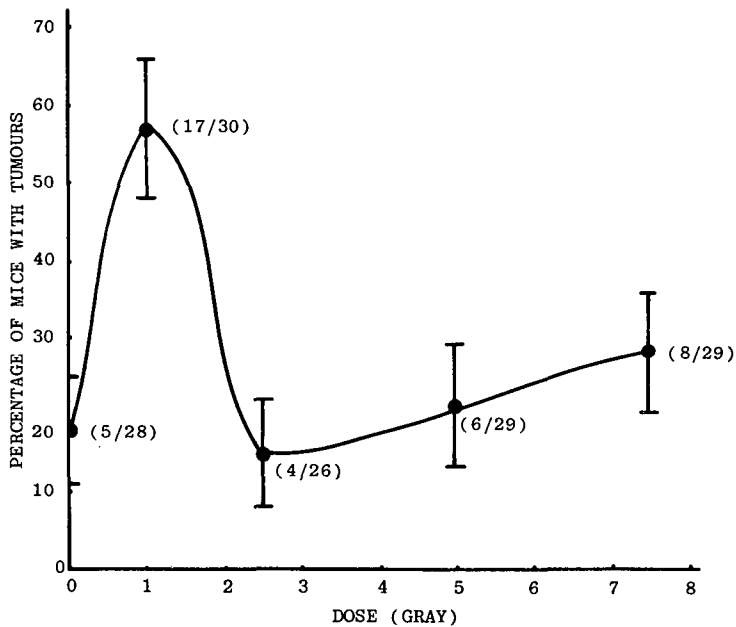


FIG.2. Percentage of mice with primary lung tumours (\pm S.E.) after 'microbeam' whole-lung irradiation. Figures in brackets indicate (number of mice with tumours)/(total number of mice in group).

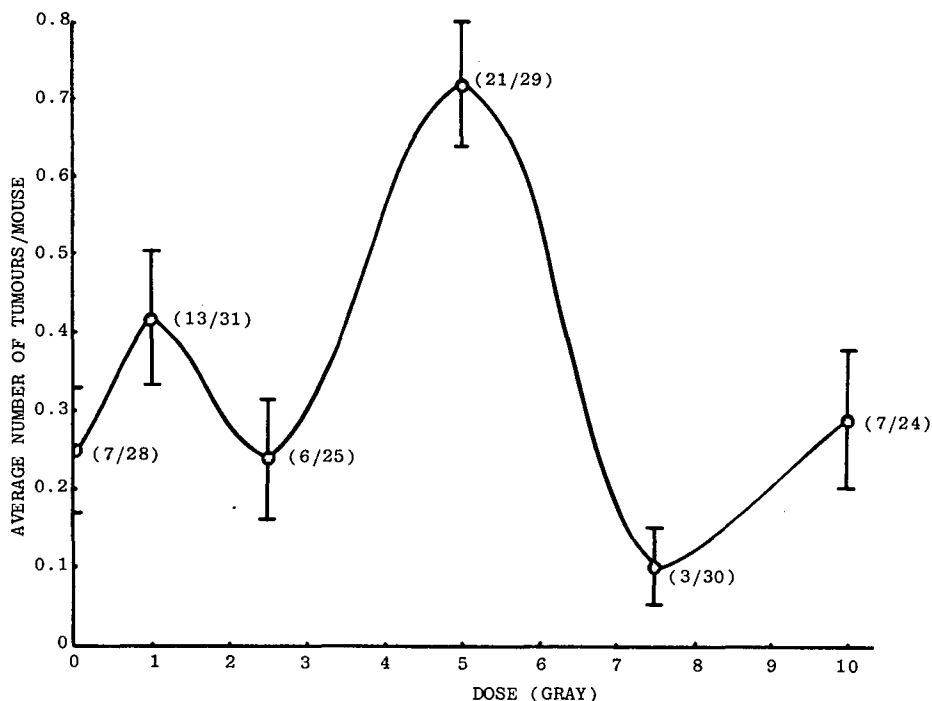


FIG. 3. Average number of tumours per mouse (\pm S.E.) after uniform whole-lung irradiation. Figures in brackets indicate (total number of tumours)/(total number of mice in group).

The absorbed doses used for uniform irradiation were 1.0, 2.5, 5.0, 7.5 and 10 Gy, and for 'microbeam' irradiation the absorbed doses were 1.0, 2.5, 5.0 and 7.5 Gy. It should be emphasised that these doses are averaged over the whole lung. Control mice were sham-irradiated. For irradiation the mice were anaesthetised with 57 mg/kg body weight sodium pentobarbitone. Afterwards the mice were caged in groups of eight, fed MRC diet 41B and given water ad lib. They were examined regularly for signs of morbidity.

Twelve months after irradiation the mice were killed with anaesthetic, exsanguinated, and the lungs inflated with formol acetic alcohol and cleared with methyl salicylate (4). This method allowed detection of tumours down to 0.5 mm diameter at any depth in the lung.

Section of all tumours were prepared for histopathological analysis.

RESULTS

Fig. 1 shows the percentage of mice with primary lung tumours after uniform whole-lung irradiation. The tumour

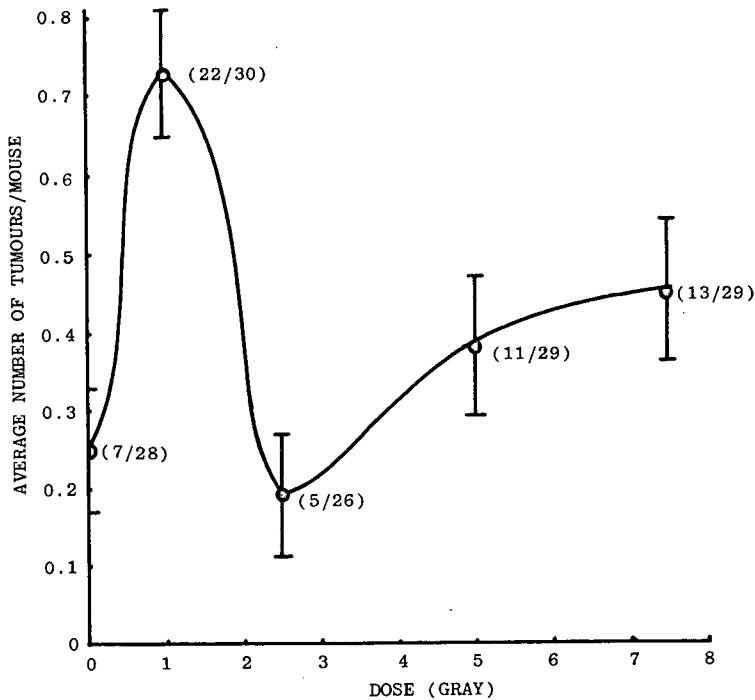


FIG.4. Average number of tumours per mouse (\pm S.E.) after 'microbeam' whole-lung irradiation. Figures in brackets indicate (total number of tumours)/(total number of mice in group).

incidence rises from a control value of $18 \pm 7\%$ to a peak of $47 \pm 9\%$ at 5 Gy. It then falls to control levels by 7.5 Gy and 10 Gy.

Fig. 2 shows the percentage of mice with primary lung tumours after 'microbeam' irradiation over the whole lung area. It shows a peak value of $57 \pm 9\%$ at 1 Gy, a fall to control values at 2.5 Gy then rises again to 28% at 7.5 Gy.

Fig. 3 shows the number of tumours per mouse for uniform irradiation. The overall shape of the curve is similar to the incidence curve (Fig. 1). Similarly Fig. 4 shows the number of tumours per mouse after 'microbeam' irradiation and here again the shape of the curve is similar to the incidence curve (Fig. 2).

In the control mice the average number of tumours per tumour-bearing mouse is 1.4, and none of the dose groups in either uniformly or non-uniformly irradiated mice gave significantly different values, except 5.0 and 7.5 Gy 'microbeam' irradiation which may produce slightly more tumours per tumour-bearing mouse (1.83 and 1.63 respectively).

TABLE I. DISTRIBUTION OF PRIMARY LUNG TUMOUR SIZES

Irradiation	Dose	No. Mice	No. Tumours	Number			Percentage		
				Small ^a	Medium ^a	Large ^a	Small	Medium	Large
Control	0	28	7	5	1	1	72	14	14
Uniform	1.0	31	13	5	4	4	38	31	31
	2.5	25	6	1	3	2	17	50	33
	5.0	29	21	6	9	6	29	43	29
	7.5	30	3	1	1	1	33	33	33
	10.0	24	7	4	1	2	57	14	29
Total		139	50	17	18	15	34	36	30
'Microbeam'	1.0	30	22	12	10	0	55	45	0
	2.5	26	5	0	4	1	0	80	20
	5.0	29	11	8	3	0	73	27	0
	7.5	29	13	5	6	2	38	46	16
Total		114	51	25	23	3	49	45	6

^a Small = <1.5 mm diameter.

Medium = 1.5 - 2.5 mm diameter.

Large = > 2.5 mm diameter.

No systematic differences in lung tumour incidence were observed between male and female mice in control or irradiated mice.

A classification of tumours (small (<1.5 mm diameter), medium (1.5 - 2.5 mm diameter), large (>2.5 mm diameter)) for each dose group is shown in Table I. Of the 50 tumours induced by uniform irradiation 34% were small, 36% medium and 30% large; whereas in the 51 tumours induced by 'microbeam' irradiation 49% were small, 45% medium and 6% large.

Histological analysis shows that the majority of primary tumours have a papillary structure with uniform columns of cuboidal or columnar cells (Figs 5 and 6). In larger tumours the papillary structure tends to disappear, the cells lose their uniformity and often the tumour appears more invasive. About 60% of the tumours appeared to be invasive and could be classified as adenocarcinomas; the remaining tumours were classified as adenomas.

The eight tumours showing intrabronchial growths were all of typical columnar structure indicating possible bronchial origin.

No correlation was found between the invasiveness of the tumours and dose for either uniform or non-uniform irradiation.

DISCUSSION

Most previous studies of radiation-induced lung tumours have involved whole-body external irradiation. Lindop and Rotblat (5) and Furth et al. (6) in radiation life-span studies showed a decreasing incidence of lung tumours as a cause of death with dose. When correction was made for intercurrent mortality Lindop and Rotblat showed no change in lung tumour incidence as a cause of death whereas Furth et al. showed an even greater decline in lung tumours as a cause of death.

Mendes (7), also using whole-body external irradiation, showed there was very little change in the incidence of bronchogenic tumours at death, whilst alveogenic tumours have a peak incidence at 1.2 Gy with a subsequent fall to below control values at greater than 4 Gy.

Ullrich et al. (8) showed there was very little change in age-corrected incidence of lung tumours at death in RFM mice after acute whole-body gamma exposures of up to 3 Gy. This work also showed evidence of a peak in lung tumour incidence at 1 Gy after either acute or protracted neutron exposures, the incidence falling to control values at 2 Gy.

None of the above studies is strictly comparable to the work reported here as in all cases the irradiation is to the whole body, and in the first two cases lung tumour incidence is only measured as a cause of death. Both Mendes (7) and Ullrich (8) do give the total lung tumour incidence at death, but Mendes does not correct this for age at death.

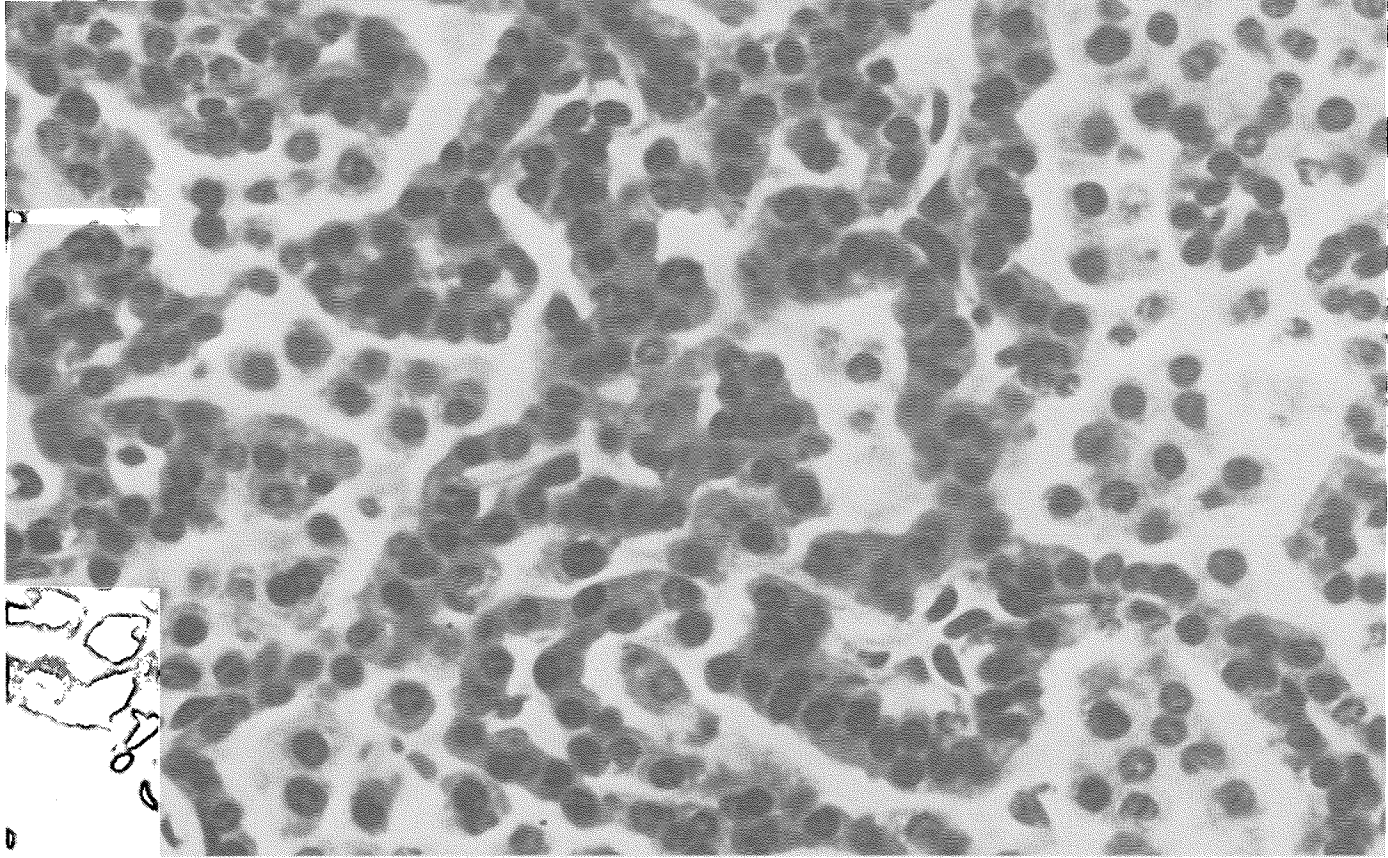


FIG.5. Primary lung tumour showing regular papillary structure of cuboidal epithelial cells (X 800).

A study that is more comparable is that of Yuhas and Walker (4) in which RF mice were given thoracic irradiation only and killed 11 months post irradiation. The dose-response curve showed a well-defined peak at 15 Gy in both lung tumour incidence and number of tumours per mouse. The shape of these curves is comparable with those obtained in these studies for uniform whole-lung irradiation (Figs 1 and 3), although the turnover point of 5 Gy in Fig. 1 is much lower than the value of 15 Gy obtained by Yuhas and Walker. It should be noted that the lowest dose used by Yuhas and Walker was 7.5 Gy, which is beyond the peak dose observed in this work, and so there is a possibility that a first peak has been missed. This discrepancy may also be due to the fact that the RF mice seem able to tolerate much higher thoracic doses than the SAS/4 mice; following 15 Gy to the whole thorax very few SAS/4 mice survive longer than 6 months.

Tumour incidence curves commonly show a turnover point, examples being for leukaemia (9), mammary tumours (10), kidney tumours (11), skin tumours (12) and lung tumours (7), (4), (8). The inflexion in these curves is usually explained by the theory that at higher doses potentially cancerous cells are killed and therefore tumour incidence is reduced (13). In vivo and in vitro experiments have shown, however, that tumour and normal cells have very similar radiosensitivities for cell-killing and therefore the same proportion of each would be killed by a radiation dose. After repopulation, providing each cell type divides in the same way, the absolute numbers of potential tumour and normal cells should be the same as if there had been no cell-killing. If increased cell-killing is to be accepted as the reason for the decreased incidence of tumours at high doses, there must be some difference in either killing or repopulation of potentially tumorous cells (14).

At present there is no other reasonable explanation for a turnover point in radiation-induced tumour incidence curves.

The difference in size-distribution of tumours between uniformly and non-uniformly irradiated mice (Table I) possibly indicates a difference in latency after the two types of irradiation; the very small proportion of large tumours after 'microbeam' irradiation suggesting an increased latency period compared with uniform irradiation. This observation is also made by Albert and Burns (15). After irradiating rat skin uniformly or through a sieve with 1.6 mm diameter holes with 25 kVp X-rays, they note a delayed increase in incidence of tumour induction in the sieve-irradiated mice.

The main finding of the work reported here is the displacement of the peak incidence of lung tumours from a value of 5 Gy after uniform irradiation to 1 Gy after non-uniform, 'microbeam' irradiation (Figs 1 and 2). The shift may be explained by considering the differences in pattern of absorbed dose. It must be remembered that the doses shown on the graphs are average doses for the whole of the lung. In the case of the 'microbeam' irradiations only approximately

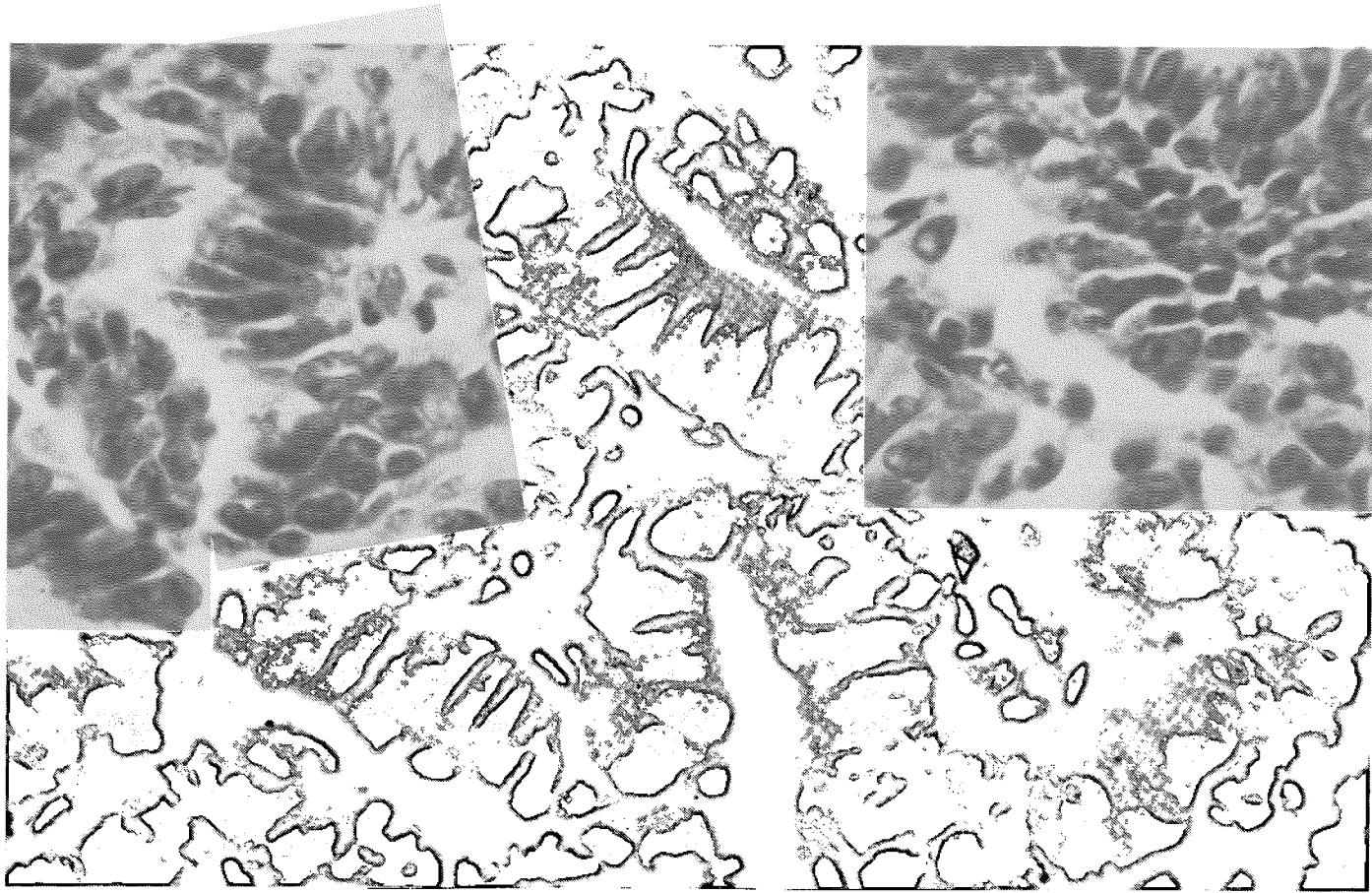


FIG. 6. Primary lung tumour showing regular papillary structure of columnar epithelial cells ($\times 800$).

20% of the lung is actually exposed, the irradiated part receiving five times the average lung dose. So a dose of 1 Gy to the lung from 72 'microbeams' involves 20% of the lung receiving a dose of 5 Gy and 80% of the lung receiving no dose. This distribution is slightly 'blurred' by the respiratory movements, but in anaesthetised mice the respiration rate is sufficiently low to cause the distribution not to differ significantly from the idealised situation.

Therefore, considering the dose to the irradiated tissue only, the peak tumour incidence occurs at the same dose of 5 Gy and the percentage incidences at this dose ($47 \pm 9\%$ for uniform, $57 \pm 9\%$ for non-uniform) are not significantly different from each other.

In a study similar to this in the rat, Bond et al. (2) produced a dose-response curve for mammary tumour induction after uniform whole-body external irradiation which had a peak at 4 Gy. Irradiation through a grid, giving an average whole-body dose of 4 Gy, gave fewer tumours than a uniform dose of 4 Gy. They concluded that the dose received by the irradiated tissue (9 Gy) was 'suboptimal' for mammary tumour production when seen in conjunction with the uniform radiation dose-response curve. The results of the present study would predict that a dose of about 2 Gy given through the 'holes' would produce the same peak incidence as did 4 Gy uniformly.

The work reported here on lungs suggests that the important factor in tumour induction is the dose absorbed by that fraction of the tissue irradiated rather than the average dose to the whole organ. The experiment does not give any information on the effect of further decreasing the volume of tissue irradiated, and extrapolation to highly non-uniform doses involving very small tissue volumes ('hot spots'), as in the case of alpha-emitters in the lung, may not be warranted.

Nevertheless, the work does indicate that the use of average tissue dose to assess risk of cancer induction may not be justifiable, and may lead to underestimation of the hazard.

ACKNOWLEDGEMENTS

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DISCUSSION

F. DEVIK: Figures 1 and 2 in your paper essentially show that the lung tumour yield is the same after 5 Gy to the whole lung as after 5 Gy to one fifth of the lung, which is rather surprising. Do you have any explanation for this?

J.E. COGGLE: I agree with you that the data are in some ways rather strange. However, they are really surprising only inasmuch as they imply that there is not the simple (expected?) relationship between the number of 'cells at risk' (i.e. the volume of tissue irradiated) and the number of lung tumours induced. In this context it may be important to remember that in both the control and the irradiated mice only one to two lung tumours per mouse are inducible. We do not see multiple tumours at any dose levels. It may be that the presence of a developing tumour in any part of the lung has a suppressive effect on other potential tumours. Since only one potential tumour cell has to be induced, there is no advantage gained by irradiation of larger and larger lung volumes. Another possible factor to consider is the complexity of the absorbed dose pattern in the 'microbeam' irradiations; this may involve a penumbra of dose that may be optimal for tumour induction with minimal killing of potential cancer cells.

B.W. WACHHOLZ: Do you intend to extend your studies to smaller volumes of irradiated lung and, if so, would you retain the same range of doses?

J.E. COGGLE: We intend to go both ways and to (1) irradiate the lung with larger pencil beams involving > 20% of the lung volume right up to hemithoracic irradiations, and (2) attempt some irradiations with smaller beams than the average 0.7 mm² beams reported here today, but we shall be severely restricted by dose-rate considerations.

We would aim to give doses such that the irradiated lung volumes receive doses two to three gray either side of 5 Gy — irrespective of average tissue dose.

INFLUENCE OF DOSE, DOSE RATE AND RADIATION QUALITY ON RADIATION CARCINOGENESIS AND LIFE SHORTENING IN RFM AND BALB/c MICE

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Abstract

INFLUENCE OF DOSE, DOSE RATE AND RADIATION QUALITY ON RADIATION CARCINOGENESIS AND LIFE SHORTENING IN RFM AND BALB/c MICE.

Over the past few years large-scale experiments in mice have been carried out on the late biological effects as a function of dose, dose rate and radiation quality. Specifically, a study has been made of the effects produced by ^{137}Cs gamma rays delivered at a high (45 rads/min) or intermediate (8.2 rads/day) dose rate, and the effect of fission neutrons at a high (25 rads/min) and low (1 rad/day) rate in a population of nearly 30 000 RFM and 11 000 BALB/c mice. Gamma-ray doses ranged from 10 to 400 rads with the RFM and from 50 to 400 rads with the BALB/c; neutron doses ranged from 5 to 200 rads with both strains. Data from these studies are now available both for life shortening and for the induction of a variety of neoplastic diseases. A survey is given of these data and the general findings; subsequent publications will present detailed analyses of each aspect. A variety of neoplasms were sensitive to induction after radiation exposure, including tumours of both reticular tissue origin (leukaemia, lymphoma, etc.) and solid tumours. For the RFM, thymic lymphomas were the dominant reticular tissue neoplasm and most of the solid tumours were either lung adenomas or fit into the broad category of endocrine-related tumours, including ovarian, pituitary, harderian and uterine tumours. The BALB/c was much less sensitive to induction of reticular tissue neoplasms. The tumours that were most sensitive to induction included malignant lung carcinomas, mammary adenocarcinomas and ovarian tumours. In general for both life shortening and tumour induction after gamma-ray exposures, when the low to intermediate dose range was sufficiently defined, linearity could be rejected, and a dose-squared or linear-dose-squared relationship adequately fits the data. For neutron exposures, on the other hand, linear relationships were the general finding. The RBE for neutrons varied with tumour type and total dose level. For gamma-ray irradiation, the intermediate dose rate resulted in a decreased effectiveness in all cases, whereas for neutron exposures the dose-rate relationships were more complex.

INTRODUCTION

Although a large amount of research has been directed towards the study of radiation-induced life shortening and carcinogenesis, many problems remain. For life shortening it can be generally concluded that low LET radiations (X- or gamma rays) are more effective when delivered at high dose rates than at low dose rates, whereas high LET radiations show less dose-rate dependence and are more effective on a dose for dose basis than are low LET radiations [1–3]. However, information on the forms of the dose-response relationships for high and low LET radiation, and quantitative information on the influence of dose rate and radiation quality are still lacking. For carcinogenesis the problems are even greater. At present there are few tumours for which the influence of dose, dose rate and radiation quality has been analysed systematically. Therefore generalizations from the data at present available are difficult.

Over the past few years experiments have been conducted in this laboratory on the influence of dose, dose rate and radiation quality on radiation-induced life shortening and carcinogenesis. Specifically we have studied the effects produced by ^{137}Cs gamma rays delivered at high and intermediate dose rates, and the effects of fission energy neutrons at a high and at a low dose rate. A survey is presented of these data and the general findings; subsequent publications will present a detailed analysis of each aspect.

MATERIALS AND METHODS

Experimental protocol

The experimental groups and the number of animals in each are shown in Tables I–III. As shown in Table I, experiment I contained both male and female RFM mice. Although the difference in response between the RFM male and female mice is of importance, the main purpose of this survey is to present data on dose-response relationships for life shortening and carcinogenesis and the influence of dose rate and radiation quality on the relationship. Since only female mice were used in the studies on dose rate and radiation quality, discussion of experiment I will be limited to the effects seen in the females. In Table III it can be seen that the acute portion of the neutron experiment actually consisted of two separate experiments. For the RFM these were not run simultaneously and differences in control incidences of thymic lymphoma were seen between these two experiments. Therefore during analysis we have treated these two portions of the RFM experiment as separate experimental groups. For the BALB/c experiment these two experiments were performed simultaneously. Since no differences were seen in these two groups they were treated as a single experimental series. The animals used in these experiments were germ-free-derived,

TABLE I. EXPERIMENT I: RADIATION DOSES AND SAMPLE SIZES OF RFM MICE EXPOSED TO GAMMA RAYS AT 45 rads/min

Strain and sex	Dose (rads)	Number of mice exposed
RFM♀♀	0 ^a	4014
	10	2827
	25	965
	50	1143
	75	246
	100	1100
	150	1043
	200	333
	300	4133
	400	396
RFM♂♂	0 ^a	430
	10	256
	25	94
	50	247
	100	230
	150	204
	300	571

^a Controls.

specific-pathogen-free, 12-week-old female RFMf/Un and BALB/c/AnNBd mice housed in the Biology Division barrier facility. Details of the maintenance of these animals and their environment have been reported previously [4, 5]. Cages were checked twice daily (5 days/week) for dead or moribund animals; these were removed and autopsied, and tissues were taken for histologic examination.

Irradiation factors and procedures

Mice receiving gamma irradiation at high dose rates were exposed in rotating individual plastic tubes to a 2000 Ci ¹³⁷Cs source at a distance of 45 cm and a dose rate of 45 rads/min. For low dose-rate exposures, a 10 Ci ¹³⁷Cs source was used. Mice were exposed at a dose rate of 8.3 rads/day for a 20-h day with the dose calculated as the dose to the midline of the cages housing the mice.

TABLE II. EXPERIMENT II: RADIATION DOSES AND SAMPLE SIZES OF RFM AND BALB/c FEMALE MICE EXPOSED TO GAMMA RAYS AT 40 rads/min OR 0.0069 rads/min

Strain and sex	Dose rate (rads/min)	Dose (rads)	Number of mice exposed
RFM ♀♀	40	0 ^a	749
		50	775
		200	766
	0.0069 ^b	50	1468
		100	1531
		200	1526
		400	866
BALB/c ♀♀	40	0 ^a	865
		50	860
		200	865
	0.0069 ^b	50	1293
		100	1323
		200	1372
		400	1013

^a Controls.

^b At this dose rate the mice received 8.3 rads/20-h exposure day.

The details of the dosimetry and exposure conditions for both the high dose-rate and low dose-rate neutron irradiations have been described previously [6, 7]. Briefly, for high dose-rate exposures, mice were exposed at the Health Physics Research Reactor (HPRR) in rotating nylon tubes. The low dose-rate exposures were made with a 1.1 mg ²⁵²Cf source surrounded by a depleted ²³⁸U sphere to reduce the gamma-ray component and to degrade the neutron spectrum to make it similar to the HPRR spectrum. Animals were exposed at a dose rate of 0.96 rads/day for a 20-h day with doses calculated at the midline of the cages housing the mice.

Statistical procedures

To correct for animals lost to follow-up through accidental death or removal, mean survival times and their standard errors were calculated by a modification of the method described by Hoel and Walburg and by a method based on Bayesian

TABLE III. EXPERIMENT III: RADIATION DOSES AND DOSE RATES AND SAMPLE SIZES OF RFM AND BALB/c FEMALE MICE EXPOSED TO FISSION NEUTRONS

Strain and sex	Dose rate	Dose (rads)	Number of mice exposed
RFM	—	0 ^a	312
	25 rads/min	24	303
		47	324
		94	333
		94 at 25 weeks	224
		188	332
	1 rad/day	24	311
		47	311
		94	309
		188	368
	5 rads/min	4.8	112
		9.6	110
		19.2	111
		47.0	112
		188	328
BALB/c	—	0 ^a	296
	25 rads/min	24	324
		47	315
		94	323
		94 at 25 weeks	224
		188	328
	1 rad/day	24	311
		47	311
		94	319
		188	360
	5 rads/min	4.8	111
		9.6	112
		19.2	112
		47.0	108
		188	360

^a Controls.

statistics [7, 8]. All regression equations were fitted to the data on mean survival times. Life shortening was calculated by subtracting the mean survival time in each experimental group from the mean survival time of the appropriate control group. The standard errors of 'days of life shortening' were obtained as the square root of the summed, squared standard errors of the two groups.

The distribution of ages at death differed considerably among the various treatment groups. Because of this, the values for observed incidence of the various neoplasms do not accurately reflect the tumorigenic effectiveness of the radiation exposures. To reflect this effectiveness more accurately, we elected to use the direct age-adjustment procedure. This procedure is a common tool of the epidemiologist which adjusts the incidence values to those that presumably would have been observed had all the groups shown the same distribution of ages at death as a common reference population. The procedures differ somewhat for diseases that are rapidly lethal and those that are slowly progressive and often seen as an incidental finding when an animal dies from some other cause. Both procedures have been presented in detail elsewhere [6, 9].

Because there were differences in control incidences of thymic lymphoma between the gamma-ray and neutron-irradiated groups and between neutron-irradiated groups, it was necessary when making comparisons in these cases to correct for the control tumour incidence using Abbott's formula [6]. These corrected incidences have only been used for drawing dose-response curves which would best show the relationship between neutron and gamma-ray exposure groups, and have not been used for analysis of the shapes of the dose-response curves.

Linear, square root of the dose, dose-squared or linear-dose-squared regression equations were fitted to the data. In all cases, the experimental values were weighted by the inverse of their calculated variance. Goodness of fit was tested using standard χ^2 tables.

RESULTS AND DISCUSSION

Dose response relationships after acute gamma-ray exposures

Sufficient data to examine in detail the form of the dose-response relationships for life shortening and carcinogenesis after acute gamma-ray irradiation are only available for the RFM strain. For life shortening (Fig.1) no simple model adequately described the entire dose range; rather at least two distinct components were seen. Over the dose range of 0–50 rads a dose-squared model adequately described the relationship ($P > 0.80$) and linearity could be rejected. Above 50 rads, although a general linear trend was observed, linearity over the 100–400 rads range could be rejected because of inflections in the curve.

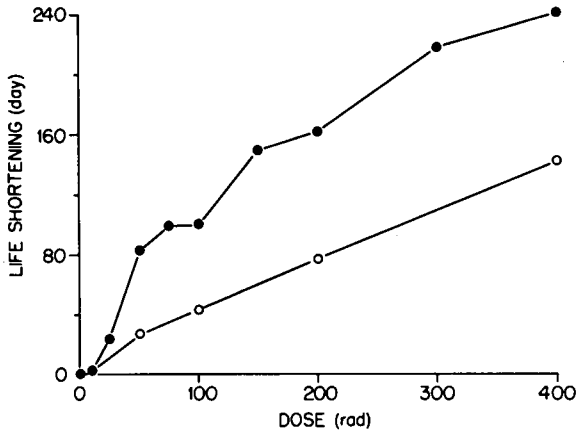


FIG.1. Life shortening as a function of dose in female RFM mice after 45 rads/min (●) or 8.3 rads/day (○).

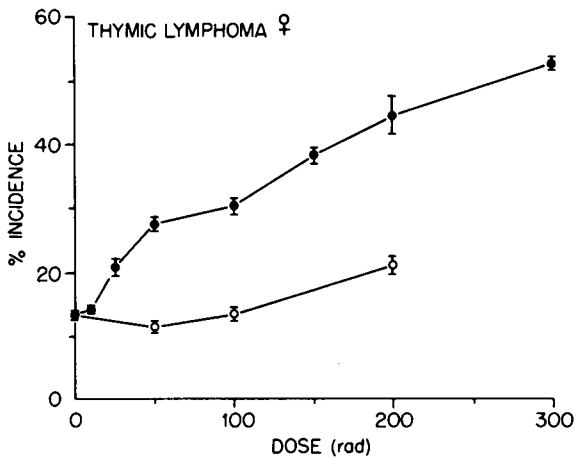


FIG.2. Percentage incidence of thymic lymphoma as a function of dose in female RFM mice after 45 rads/min (●) or 8.3 rads/day (○).

A number of neoplasms were sensitive to induction after radiation exposure, including neoplasms of reticular tissue origin and solid tumours. For the RFM female, thymic lymphoma and reticulum cell sarcoma were the dominant reticular tissue neoplasms. As has been reported previously, a decrease in reticulum cell sarcoma was observed after both gamma-ray and neutron exposures [6, 10].

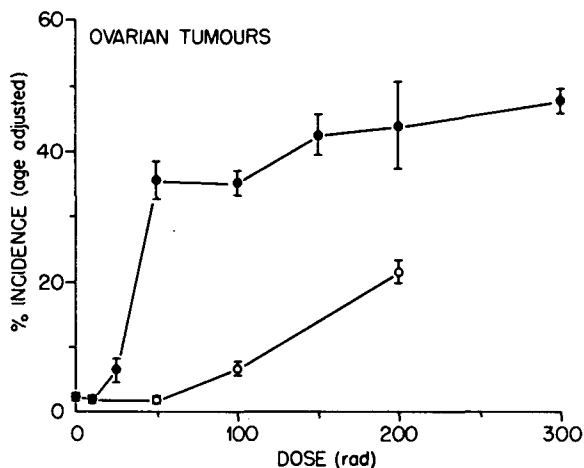


FIG. 3. Age-adjusted percentage incidence of ovarian tumours as a function of dose in female RFM mice after 45 rads/min (●) or 8.3 rads/day (○).

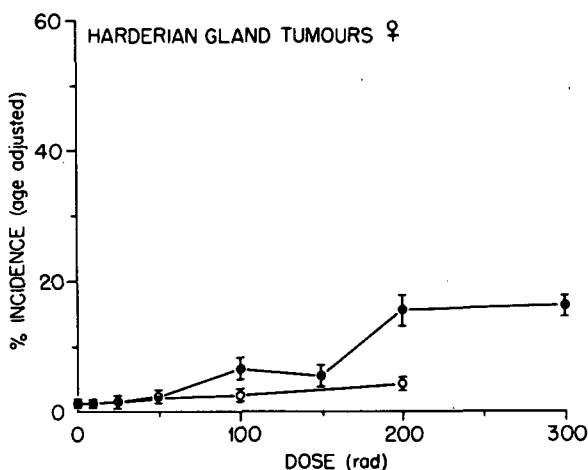


FIG. 4. Age-adjusted percentage incidence of harderian gland tumours as a function of dose in female RFM mice after 45 rads/min (●) or 8.3 rads/day (○).

The dose-response relationship for thymic lymphoma after acute gamma-ray exposures is shown in Fig. 2. As with life shortening, no simple relationship adequately described the form of the entire dose-response curve. Over the limited range of 0–25 rads, linearity could be rejected ($P < 0.01$), and a dose-squared model adequately described the relationship. Over the 50–300 rads range the observed increase in incidence with dose was more nearly linear. The mechanistic basis for this apparent two-component curve is not known.

Most of the solid tumours in the RFM female fit into the broad category of endocrine-related tumours, including ovarian, pituitary, harderian and uterine. A number of lung adenomas were seen in all groups, but no significant increases in incidence over control values were detected at any dose except at 300 rads. Two types of solid tumours, ovarian and harderian gland, shown in Figs 3 and 4 respectively, generally serve to illustrate the dose responses observed for the induction of tumours in the female RFM. Ovarian tumours were quite sensitive to induction. Over the dose range of 0–50 rads a linear quadratic relationship adequately described the relationship, and both linear and dose-squared models could be rejected ($P < 0.01$). Following the rapid increase in incidence between 0 and 50 rads, a more gradual linear increase was observed. Most other tumours were not as sensitive to induction after acute gamma-ray exposures as thymic lymphoma and ovarian tumours. Rather the dose-response relationships for uterine, pituitary and harderian gland tumours were all quite similar, and could be illustrated by the dose-response relationships for harderian gland tumour inductions shown in Fig.4. For this tumour, a linear quadratic model adequately describes the relationship between dose and tumour induction over the dose range of 0–200 rads. Over this same range a linear model could be rejected ($P < 0.05$).

Dose-response relationships after intermediate dose-rate gamma-ray exposure

Because RFM mice are known to be sensitive to the induction of reticular tissue neoplasms, in the studies on dose rate and radiation quality the BALB/c strain was used in addition to the RFM in order to obtain more information on the induction of solid tumours. A comparison of the extent of life shortening in the RFM after high dose-rate and intermediate dose-rate gamma-ray exposures is shown in Fig.1. After intermediate dose-rate gamma-rays a linear relationship passing through the intercept adequately described the data. The large difference in effectiveness of the radiation at the two rates seemed to be due primarily to an upward displacement of the regression line at the high dose rate which seems to take place over the 0–50 rads dose range after high dose-rate exposures.

The more limited data comparing high and intermediate dose-rate effects in BALB/c females were consistent with the RFM data. As shown in Fig.5, the major difference in the two curves is in the upward displacement at the high dose rate which has occurred over the 0–50 rads range. These data suggest a highly sensitive dose-rate-dependent injury component for life shortening in both the RFM and BALB/c which presumably saturates at high dose rates at about 50 rads. This component may be similar to the 'dose independent component of radiation mortality in female mice' earlier identified by Sacher in mice given 200 R or more [11].

For tumour induction in the RFM female the lower gamma-ray dose rate was significantly less effective in all cases. Surprisingly, for thymic lymphoma

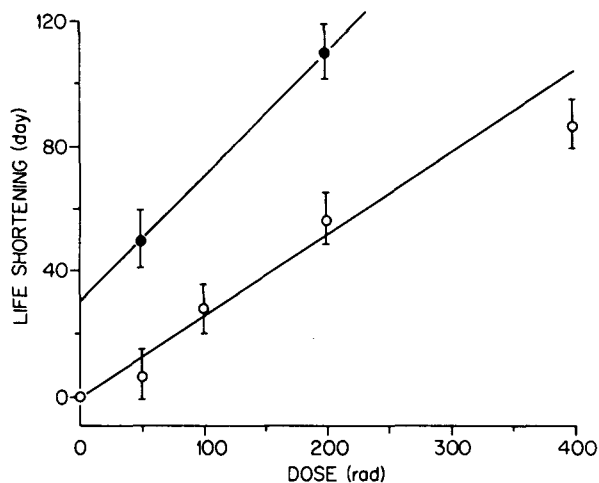


FIG. 5. Life shortening as a function of dose in female BALB/c mice after 45 rads/min (●) or 8.3 rads/day (○).

TABLE IV. INFLUENCE OF DOSE RATE ON INDUCTION OF NEOPLASTIC DISEASES IN FEMALE BALB/c MICE EXPOSED TO GAMMA RAYS

Tumour	Age-adjusted incidence (%±S.E.)		
	Dose (rads)	High dose rate ^a	Low dose rate ^b
Ovarian tumours	0	6.4 ± 1.4	
	50	66.1 ± 2.3	9.9 ± 1.8
	100	—	21.9 ± 2.6
	200	75.9 ± 2.2	42.5 ± 2.8
Mammary adenocarcinomas	0	7.6 ± 0.9	
	50	12.1 ± 1.4	9.0 ± 0.9
	100	—	13.2 ± 1.2
	200	20.5 ± 2.5	13.9 ± 1.3
Lung adenocarcinomas	0	12.8 ± 2.2	
	50	21.4 ± 3.0	14.5 ± 1.8
	100	—	16.5 ± 2.1
	200	36.8 ± 5.4	21.4 ± 2.6

^a 45 rads/min.

^b 8.3 rads/day.

and ovarian tumours, the relationships between tumour induction and dose shown in Figs 2 and 3 respectively, were best described by a linear quadratic model and linearity could be rejected ($P < 0.001$) even at this lower dose rate. For harderian gland tumours (Fig.4), dose response at the lower dose rate was linear ($P > 0.90$).

The more limited data comparing the effects of high and intermediate dose-rate gamma-ray exposures on tumour induction in female BALB/c mice are shown in Table IV. The BALB/c were much less sensitive to the induction of reticular tissue neoplasms. The tumours that were most sensitive to induction included malignant lung adenocarcinomas, mammary adenocarcinomas and ovarian tumours. As in the RFM, the lower dose rate was less effective in all cases. Also as in the RFM, the dose-response relationship for the induction of ovarian tumours at the lower dose rate was adequately described by a linear quadratic model and linearity could be rejected ($P < 0.05$). For mammary and lung adenocarcinomas, the dose-response relationships at the lower dose rate could be adequately described by the linear relationships:

$$y = 7.8 + 0.035X$$

and

$$y = 12.5 + 0.043X$$

respectively. Since the limited high dose-rate data for mammary and lung tumours were also adequately described by linear relationships:

$$y = 7.9 + 0.067X$$

and

$$y = 13.4 + 0.12X$$

and since the intercepts of the equations were similar, the differences in slope were a reflection of differences in effectiveness of the two dose rates. The ratio of the slope constants for mammary tumours ($[0.067/0.035] = 1.9$) and lung tumours ($[0.12/0.043] = 2.8$) suggest a greater dose-rate effect for lung tumour induction than for the induction of mammary tumours.

Influence of radiation quality on dose-response relationships for life shortening and carcinogenesis

After high dose-rate neutron irradiation of both RFM and BALB/c females, the dose response for life shortening over the dose range including 0, 24, 47 and 94 rads was adequately described by regression of survival time as the square root of the dose (Figs 6 and 7). However, for the data covering the dose range of 0–47 rads shown in Table V a square-root regression could be rejected ($P < 0.01$), and linearity gave a good fit for both the RFM ($P > 0.9$) and the

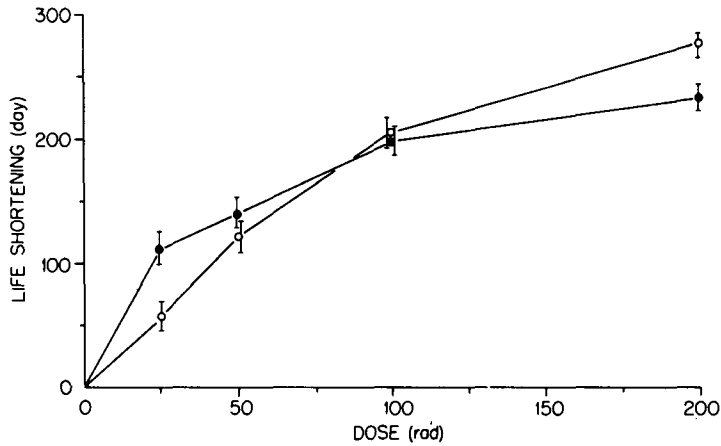


FIG. 6. Life shortening as a function of dose in female RFM mice after irradiation with neutrons at a high (●) or low (○) dose rate.

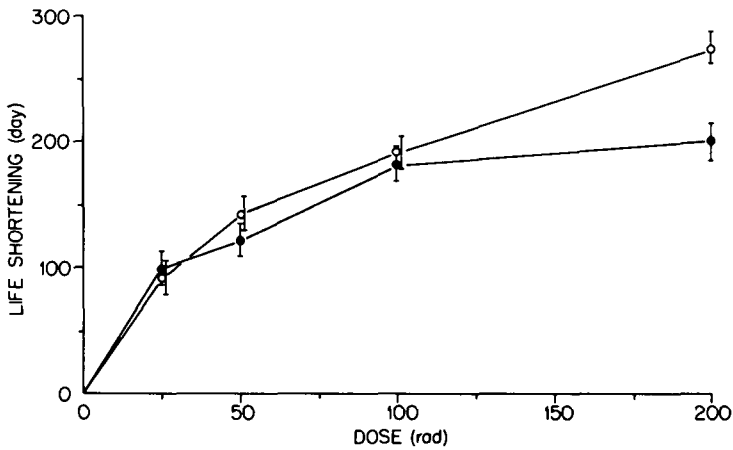


FIG. 7. Life shortening as a function of dose in female BALB/c mice after irradiation with neutrons at a high (●) or low (○) dose rate.

BALB/c ($P > 0.5$). After low dose-rate neutron irradiation, the response was somewhat different. In RFM mice, the low dose rate was less effective than the high dose rate at 24 rads, but more effective at 188 rads (Fig. 6). In the BALB/c, little dose-rate dependence was observed at low doses, whereas at the 188 rads dose the low dose rate was more effective (Fig. 7). The greater life shortening at 188 rads with low dose-rate neutron exposure is consistent with previous

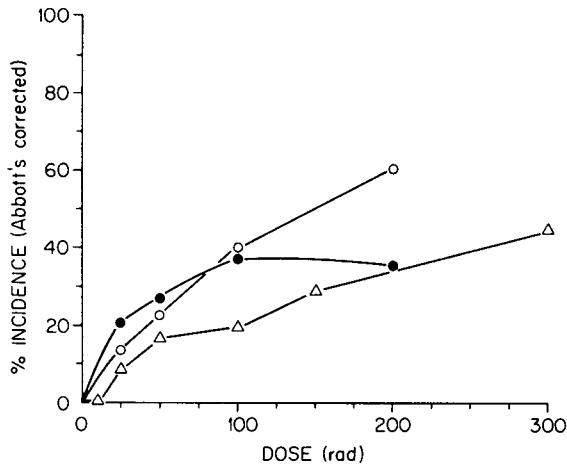


FIG. 8. Percentage incidence of thymic lymphoma after acute gamma-ray (Δ), acute neutron (\bullet), or chronic neutron (\circ) irradiation.

TABLE V. MEAN AGES AT DEATH OF RFM AND BALB/c FEMALE MICE EXPOSED TO FISSION NEUTRONS

Strain and sex	Dose rate	Dose (rads)	Number of mice exposed	Mean age at death (days \pm S.E.)	Life shortening (days \pm S.E.)
RFM	—	0 ^a	312	644.0 \pm 8.21	—
	5 rads/min	4.8	112	620.0 \pm 15.32	24.0 \pm 17.38
		9.6	110	616.2 \pm 14.63	27.8 \pm 16.78
		19.2	111	588.0 \pm 16.97	56.0 \pm 18.85
		47.0	112	486.4 \pm 16.35	157.6 \pm 18.30
BALB/c	—	0 ^a	296	794.8 \pm 9.02	—
	5 rads/min	4.8	111	795.1 \pm 16.28	— 0.3 \pm 18.61
		9.6	112	746.9 \pm 16.82	47.9 \pm 19.09
		19.2	112	686.5 \pm 16.29	108.3 \pm 18.62
		47.0	108	638.5 \pm 15.77	156.3 \pm 18.17

^a Controls.

TABLE VI. INCIDENCE OF THYMIC LYMPHOMA IN FEMALE RFM MICE AFTER NEUTRON IRRADIATION

Type of neoplasm	Incidence (%±S.E.) after a dose (rads) of:				
	0 (control)	4.8	9.6	19.2	47
Thymic lymphoma	7.3 ± 1.2	10.9 ± 2.7	10.9 ± 2.1	19.8 ± 3.0	33.0 ± 3.3

observations [12]. The reduced effectiveness at 24 rads in the RFM mice was more surprising. Since the result was obtained at one dose and in only one of the strains, it might be argued that this was a spurious point or may reflect some effect unique to the RFM female mouse. However, recent data from the Argonne National Laboratory also suggest such a reduced effect at a dose of 20 rads when the dose was fractionated [13]. Such data suggest that further studies examining the effects of low dose-rate neutron irradiation below 20 rads are warranted.

Irradiation with neutrons was also more effective in inducing tumours than was irradiation with gamma rays, particularly in the low dose range. For the RFM, because of the small sample sizes used in the experiment examining the 0, 4.8, 9.6, 19, 24 and 47 rads range, solid tumours could not be analysed and information in the low range was only available for the induction of thymic lymphoma. The data from the larger experiment covering the range of 0, 24, 47, 94 and 188 rads are shown in Fig.8. For the acute neutron dose-response curve over the 0–94 rads range linearity could be rejected ($P < 0.001$), and a dose-squared model adequately described the data. The 0–47 rads range was able to be examined more fully by using the data for thymic lymphoma from the smaller experiment. Although the control incidence in this smaller experiment was slightly different than in the larger experiment, correction with Abbott's formula indicated that the excess incidence was similar at similar doses for both experiments. Because of this we felt that the data from the smaller experiment would be applicable for defining the shape of the dose-response curve. Using the data shown in Table VI a linear relationship adequately described the curve over the 0–47 rads range ($P > 0.75$):

$$y = 5.2 + 0.56X$$

In the chronically exposed group a linear relationship adequately described the curve over the 0–94 rads range ($P > 0.8$):

$$y = 5.3 + 0.39X$$

TABLE VII. INCIDENCE (%±S.E.) OF SOLID TUMOURS IN NEUTRON-IRRADIATED BALB/c FEMALE MICE

Dose rate	Dose	Tumour type		
		Lung adenocarcinoma	Mammary tumours	Ovarian tumours
5 rad/min	0 (control)	12.8 ± 3.4 ^a (12.8) ^b	7.0 ± 1.6 (7.0)	5.5 ± 2.1 (5.5)
	4.8	27.1 ± 4.8 (27.5)	6.9 ± 2.6 (7.7)	6.7 ± 4.1 (5.0)
	9.6	38.7 ± 5.1 (29.2)	24.8 ± 4.5 (20.6)	10.5 ± 4.6 (8.3)
	19.2	19.3 ± 5.1 (11.3)	17.8 ± 5.0 (11.6)	19.7 ± 4.9 (25)
	47	21.7 ± 4.7 (13.6)	17.2 ± 5.6 (8.4)	49.2 ± 4.0 (45.5)
25 rad/min	24	19.3 ± 4.8 (12.2)	16.9 ± 2.4 (9.0)	37.1 ± 4.6 (34.6)
	47	22.7 ± 5.1 (13.5)	18.9 ± 3.7 (9.2)	56.8 ± 5.4 (57.6)
	94	18.6 ± 5.7 (8.0)	16.9 ± 3.9 (9.3)	61.5 ± 3.5 (47.9)
	94 at 25 weeks	20.0 ± 5.2 (10.1)	17.2 ± 4.9 (11.6)	43.0 ± 5.2 (42.5)
	188	12.5 ± 5.2 (5.0)	15.3 ± 5.4 (5.0)	38.5 ± 5.5 (21.3)
1 rad/day	24	12.5 ± 4.6 (8.9)	13.5 ± 2.9 (8.3)	6.7 ± 2.9 (6.6)
	47	26.9 ± 5.7 (13.1)	17.2 ± 3.7 (8.9)	9.6 ± 3.7 (6.9)
	94	31.6 ± 5.5 (13.8)	19.2 ± 3.8 (10.2)	18.6 ± 4.2 (19.1)
	188	43.1 ± 5.7 (15.6)	45.2 ± 5.3 (17.0)	20.9 ± 5.1 (6.0)

^a Age-adjusted incidence.^b Observed incidence.

The ratio of the slope constants suggests that chronic exposure was less effective in the low dose range, but this difference was not significant. In the high dose range chronic irradiation was significantly more effective than acute neutron irradiation for the induction of thymic lymphoma.

The effects of dose and dose rate on tumour induction after neutron exposure in female BALB/c mice are shown in Table VII. Lung adenocarcinomas were quite sensitive to induction with neutrons with the incidence increasing rapidly with dose to a peak incidence of 38.7 at 10 rads. This curve could be adequately described by a linear dose-response relationship ($y = 12 + 2.6X$) over the 0–10 rads dose range. The dose response for the chronic neutron exposure was somewhat different, and a linear relationship adequately described the curve over the entire dose range:

$$y = 12.8 + 0.17X$$

Because of the nature of the two dose-response relationships, low dose-rate neutron irradiation appeared to be less effective than high dose-rate irradiation at low total doses, but more effective at high total doses. The data for mammary tumour induction were quite similar to those for malignant lung tumours with a rapidly rising neutron dose response over the 0–10 rads range with a linear slope of 1.14 for acute exposures. The low dose rate appeared to be less effective at low total doses, but markedly more effective than acute exposures at 188 rads. For the low dose-rate exposures a linear dose-response relationship ($y = 7.3 + 0.18X$) adequately described the dose response over the 0–188 rads range.

Neutron irradiation at low dose rates was less effective than at high dose rates in inducing ovarian tumours at all doses tested. For acute neutron irradiation the dose response could be adequately described by a linear ($P > 0.750$) ($y = 4.4 + 0.87X$) or a linear quadratic model ($P > 0.975$) ($y = 5.2 + 0.52X + 0.0072X^2$) whereas for chronic neutron irradiation a linear model ($P > 0.75$) ($y = 4.8 + 0.13X$) adequately described the relationship.

The relative biological effectiveness (RBE) of neutrons varied with the end-point and in some instances with dose level. At high dose rates of neutrons and gamma rays, the RBE for life shortening in the RFM female varied with the level of effect as indicated by the dissimilar shapes of the dose-response curves. Calculation of RBE was further complicated because of the apparent two-component nature of the life-shortening response. In the dose range below 50 rads of gamma rays it was found that the RBE of neutrons varied with the inverse of the square root of the dose. Such a relationship has been predicted by the theory of dual radiation action, and has previously been reported for a variety of end-points by Kellerer and Rossi [14]. At doses above 50 rads, an estimate of RBE, based on the ratio of slope of the linear regression of gamma-ray and neutron responses, was obtained. Because of limited data, a similar procedure was used

for the RBE estimates in the BALB/c. In this dose range the RBE estimates for RFM mice (RBE = 2.9) and BALB/c mice (RBE = 3) were remarkably similar.

Estimates of RBE values for tumour induction were also obtained. For the induction of thymic lymphoma in the RFM mouse, over the dose range of 0–50 rads the response to gamma rays varied with the square of the dose whereas the response to neutrons varied linearly. Thus, as with life shortening, the RBE increased with decreasing dose in a manner proportional to the inverse of the square root of the neutron dose. The exact relationship ($\text{RBE} = 20D_n^{-0.5}$) would predict an RBE of 4 at 25 rads and 20 at 1 rad. For the other tumours examined the dose-response relationships were less well defined, and the relationship between neutron dose and RBE could not be properly examined. For lung adenocarcinomas and mammary adenocarcinomas, because the dose-response relationships for both gamma rays and neutrons could be described by linear relationships, a comparison of the slope constants provided an estimate of the RBE for neutrons. For lung adenocarcinomas this method gave an estimated RBE of 21.7 and the RBE estimate obtained for mammary tumours using this method was 17. For ovarian tumours, because of limited information on the gamma-ray dose-response relationship in the region between 0 and 50 rads, no RBE could be estimated. At 50 rads, however, the RBE appeared to be close to one.

ACKNOWLEDGEMENTS

To accomplish experiments of this size requires the help of a great many people. It is impossible here to mention each person who made significant contributions. We would, however, like to thank J.A. Auxier and J.W. Poston for their continuing advice and support of the neutron studies, and D.J. Christian and J.H. Thorngate for performing the dosimetry on the ^{252}Cf source. We had many useful discussions on statistical methods with T.J. Mitchell. Many past and present members of the Pathology Unit, Biology Division, have made invaluable contributions, particularly G.E. Cosgrove and N.K. Clapp. Most importantly, we wish to express appreciation to A.C. Upton under whose leadership the first experiment was initiated.

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DISCUSSION

T. SADO: From the early work of A.C. Upton, it was my understanding that RFM is the strain that develops myeloid leukaemia following exposure to radiation, but you did not mention myeloid leukaemia in your presentation. Would you like to comment on this?

R.L. ULLRICH: The studies of Upton were performed with animals housed in conventional animal facilities. With specific pathogen-free animals the incidence of myeloid leukaemia is greatly reduced, particularly in females. An increase is still seen in males, although it is still not as great as was reported by Upton, but very little increase is seen in females after irradiation.

C. STREFFER: With the tumours for which you postulate a quadratic-linear dose relationship, you found that the dose rate had a strong effect on the rate of tumour induction. In contrast, with those tumours for which you postulate a linear dose relationship, you found that dose rate had only a slight effect. Can you explain this difference?

R.L. ULLRICH: I assume you are referring to the large dose-rate effects for thymic and ovarian tumours. It appears that these tumours are highly dependent on factors such as cell killing and/or hormonal status for expression. For these tumours the dose-rate effect may be marked because of the effects on cell killing and hormone levels which modify expression, whereas for the other tumours, such as lung and harderian gland, the dose-rate effect may be more directly related to carcinogenic effects rather than modifying factors.

IMMUNOLOGICAL COMPETENCE OF AGING MICE EXPOSED TO X- OR GAMMA RAYS DURING YOUNG ADULTHOOD

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Abstract

IMMUNOLOGICAL COMPETENCE OF AGING MICE EXPOSED TO X- OR GAMMA RAYS DURING YOUNG ADULTHOOD.

Male BC3F₁/Cum and B6C3F₁/Nrs mice were exposed to X-rays ranging from 150 R to 450 R at the age of 11–12 weeks. Three to 18 months later the mice were tested individually for various immunologic functions. These included: (a) antibody response of intact mice to a T-cell-dependent antigen, sheep red blood cells (SRBC); (b) ability to reject allogenic (B10.A/Sn; H-2^a) skin grafts; (c) counts of T and B lymphocytes recovered from individual spleens; (d) proliferative responses of splenic lymphocytes to a T-cell-specific mitogen, PHA, as well as to a B-cell-specific mitogen, LPS, in vitro; and (e) reactivity of splenic lymphocytes to allogenic (B10.D2/Sn; H-2^d) lymphocytes in vitro. The results indicated that under the experimental conditions employed few, if any, defects were demonstrated for most, if not all, of the immune functions examined. The only exception was the alloantigen reactivity of splenic lymphocytes examined at 18 months after exposure. In this case, a dose-dependent suppression was clearly indicated. However, in a more recent experiment with specific pathogen-free C3Hf/HeMsNrs male mice, we were unable to demonstrate a suppression of alloantigen reactivity of splenic lymphocytes even at 18 months after the whole-body exposure to 143 R — 572 R gamma rays. The possible reason for this apparent discrepancy is discussed.

1. INTRODUCTION

It is now well established that immune functions decline with age and that the lowered immune functions are inversely correlated with the higher risk of

the aged to infectious diseases, autoimmune disorders as well as neoplastic diseases [1–5]. It is also known that the immune system is highly radio-sensitive [6–8]. Thus, for example, suppression of the antibody response in mice could be demonstrated by as low as 50 R of X- or gamma rays. However, when radiation doses given to animals are not very high, the immune competence of the irradiated animals eventually recovers almost to the level of the non-irradiated age control animals within 1–3 months following the exposure, the time required for complete recovery being variable depending upon the radiation dose and the age of animals at the time of the radiation exposure. A question then arises as to the time course of the senescence of the fully restored immune system of the exposed animals: Is there any evidence of acceleration of aging of the immune system as a result of earlier exposure to radiation? It is possible, for example, that the depletion of the pool size of immunocompetent progenitor cells occurs earlier in the irradiated animals because they may have exhausted the quota of cell divisions allowed for each stem cell for immunocompetent cells [1]. Thus, it is of particular importance to know whether the life-shortening effect of radiation [9–15] could be mediated through the accelerated aging of the immune system. In view of these considerations, we undertook, several years ago, a preliminary study on the late effects of radiation on the immune system of mice. Several immunologic indices were examined. These included: (a) antibody response to a T-cell-dependent antigen, sheep erythrocytes (SRBC); (b) ability to reject allogenic skin grafts; (c) counts of T and B lymphocytes recovered from individual spleens; (d) proliferative responses of splenic lymphocytes to a T-cell-specific mitogen, PHA, as well as to a B-cell-specific mitogen, LPS, *in vitro*; and (e) reactivity of splenic lymphocytes to allogenic lymphocytes *in vitro* (mixed lymphocyte reaction, MLR). The results of this analysis are presented here.

2. MATERIALS AND METHODS

2.1. Mice

Experimental mice employed in this study were either BC3F₁/Cum ([C57BL/Cum ♀ × C3H/Cum ♂]F₁) or B6C3F₁/Nrs ([C57BL/6JNrs ♀ × C3H/HeMsNrs ♂]F₁) males, both possessing H-2^{b/k} haplotypes. The former were purchased from Cumberland View Farms, Clinton, Tennessee, United States of America. The latter were bred in our own colonies at the Animal Production Facility of this institute. The mice were housed 5 per cage in 17 × 30 × 11 cm metal cages with wood shavings as bedding. This bedding was changed weekly. Standard Purina Laboratory Chow (Funabashi Farm) and chlorinated water were given *ad libitum*.

2.2. Irradiation

Experimental mice were exposed at the age of 11–12 weeks to 150 R–450 R X-rays (Shimadzu Shinai 250 X-ray generator operating at 200 kVp, 20 mA; 0.5 mm Cu + 0.5 mm Al filters; focus-skin distance of 50 cm; dose-rate at 85 R/min in air).

2.3. Antibody response

At various intervals after irradiation, groups of mice were injected ip with 2.5×10^8 SRBC. Four days later the spleens of these mice were examined for counts of direct haemolytic plaque-forming cells (PFC).

2.4. Skin grafting

Allogenic skin used for grafting was derived from the 3-month-old B10.A/Sn (H-2^a) strain.

2.5. Counts of T and B lymphocytes

Detection and scoring of T and B lymphocytes recovered from the spleens of each test mouse were based on the immunofluorescence staining of surface markers characteristic of each cell type, i.e. Thy 1.2 antigens for the former and surface immunoglobulins for the latter. Details of the method routinely used in this laboratory have been described previously [16].

2.6. Lymphocyte culture

In vitro lymphocyte cultures were performed in microplate cultures using Falcon Microtest II plates [17]. For tests of mitogen responsiveness, 5×10^5 viable splenic mononuclear cells were cultured either with 0.5 μ g of PHA-P (Wellcome Reagents Ltd.) or with 0.5 μ g of LPS-B (*E. coli* 055:B5, Difco Laboratories) in 0.2 ml volume of medium RPMI 1640 (Nissui) containing 1% human serum. Control cultures contained no mitogen. The cultures were incubated at 37°C in a humid atmosphere of 5% CO₂ and 95% air for 48 hours, then 0.25 μ Ci of ³H-thymidine (³H-TdR; sp. act., 5.0 mCi/mM, The Radiochemical Centre) in a volume of 5 μ l was added to each culture well, and 24 hours later the cells were harvested on glass fibre filters and then processed for scintillation counting. The counts were determined in a Beckman liquid scintillation counter (Model LS-250).

For tests of alloantigen reactivities, 10^6 viable splenic mononuclear cells were cultured together with an equal number of 5000 R-treated spleen cells

(stimulator cells) derived from the B10.D2/Sn (H-2^d) strain. Control cultures contained equal numbers of similarly treated syngenic (B6C3F₁/Nrs) spleen cells. Culture conditions as well as subsequent procedures were essentially the same as those used for tests of mitogen responsiveness except that ³H-TdR was added at 72 hours and the cells harvested at 96 hours.

The degree of mitogen responsiveness as well as alloantigen reactivity was expressed by the net increase in the uptake (Δ counts/min) of ³H-TdR by the stimulated cultures compared with that of non-stimulated control cultures, or by the log stimulation ratios, e.g. $\log(^3\text{H-TdR uptake by stimulated cultures} / ^3\text{H-TdR uptake by non-stimulated control cultures})$ or $\log(^3\text{H-TdR uptake by stimulated cultures}) - \log(^3\text{H-TdR uptake by non-stimulated cultures})$.

3. RESULTS

3.1. Late effects of radiation on antibody response

Groups of BC3F₁/Cum male mice were exposed to 200 R or 400 R X-rays in one experiment (LE 731) or to 150, 300 or 450 R in another experiment (LE 732). Six, 12 and 18 months later these mice were tested individually for antibody-forming response to SRBC. The results are shown in Fig. 1. It can be seen from this figure that, at any time intervals and dose levels studied, the antibody response of the treated mice was not significantly different from that of age-matched, non-irradiated control groups.

3.2. Late effects of radiation on the skin allograft resistance

The results of one experiment in which the late effects of radiation on the skin allograft resistance of BC3F₁/Cum mice was tested are summarized in Fig. 2. From this figure it is clear that the mean survival time of the B10.A skin grafted on BC3F₁/Cum mice was not affected at all when tested 16 months after whole-body exposure to 200 R or 400 R X-rays.

3.3. Late effects of radiation on the counts of T and B lymphocytes in the spleens

Counts of T and B lymphocytes recovered from individual spleens of B6C3F₁/Nrs mice exposed to 200 or 400 R X-rays 18 months earlier are shown in Fig. 3 together with the results obtained with appropriate age control groups. They indicate: (a) A dose-dependent rise in the number of nucleated cells recovered from the spleens; the difference between the age control and the 400 R-treated group was significant ($0.01 < P < 0.05$). (b) There was no significant difference in the number of T and B lymphocytes between the irradiated and non-irradiated age control groups.

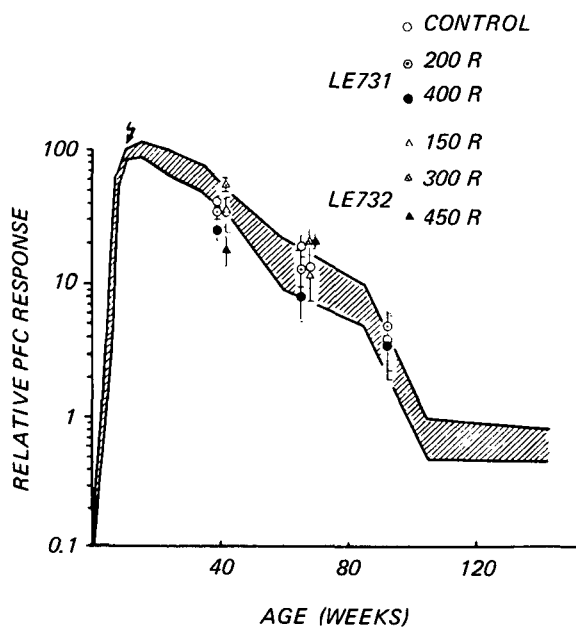


FIG.1. Late effects of radiation on the antibody-forming response of $BC3F_1/Cum$ male mice exposed to various doses of X-rays at the age of 11–12 weeks (see Section 2 for details).

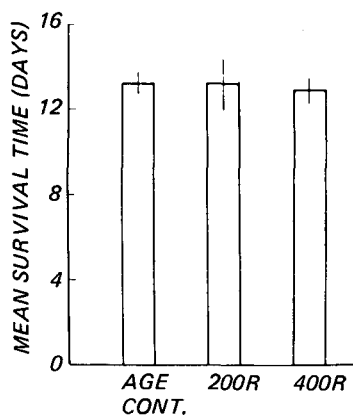


FIG.2. Mean survival time of $B10.A (H-2^a)$ skins grafted on $BC3F_1/Cum$ mice ($H-2^{b/k}$) 14 months after exposure to 200 R or 400 R X-rays.

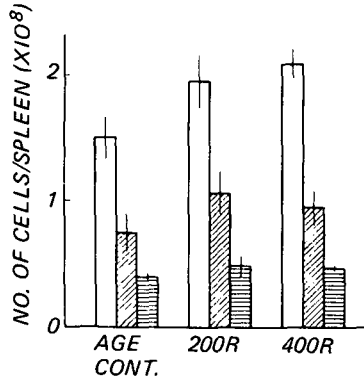


FIG.3. Numbers of nucleated cells, T and B lymphocytes recovered from individual spleens 18 months after exposure to 200 R or 400 R X-rays: □ nucleated cells; ▨ T cells; ▤ B cells.

3.4. Mitogen responsiveness of splenic lymphocytes as a function of time after exposure to X-rays

Spleen cell preparations obtained from individual B6C3F₁/Nrs mice exposed to 200 or 400 R X-rays at the age of 11–12 weeks were tested for ability to respond to PHA or LPS by proliferation *in vitro*. The mitogen responsiveness was assessed 3, 6, 12 and 18 months after the exposure. The results are shown in Figs 4 and 5. It can be seen from these figures that there was no significant difference in the responsiveness of splenic lymphocytes to PHA as well as to LPS among the irradiated and non-irradiated age control groups, at any time intervals examined. It is also noted that ³H-TdR uptake of non-stimulated spleen cell cultures increased slightly for both irradiated and non-irradiated groups in the mice aged over 1 year, suggesting an increased rate of proliferation of spleen cells in the aging mice.

3.5. Late effects of radiation on the alloantigen reactivity of splenic lymphocytes

The splenic lymphocytes used in the above experiments were also tested for ability to respond to 5000 R-treated allogenic lymphocytes *in vitro*. The results indicated that there was no effect of radiation on the alloantigen reactivity of splenic lymphocytes when tested up to 12 months following exposure. However, when the test was performed at 18 months after irradiation, a clear-cut dose-dependent decline in this reactivity was noted (Fig.6). The difference between the 400 R-treated and the age control group was highly

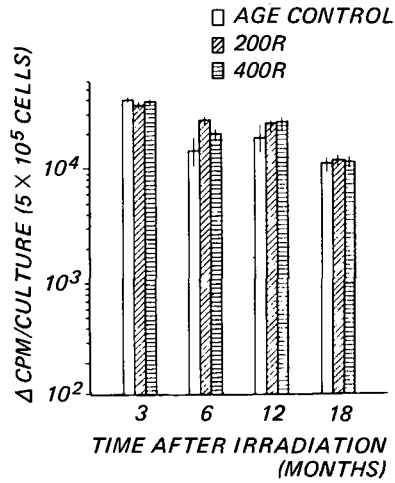


FIG. 4. PHA responsiveness of splenic lymphocytes of B6C3F₁/Nrs mice as a function of time after exposure to 200 R or 400 R X-rays.

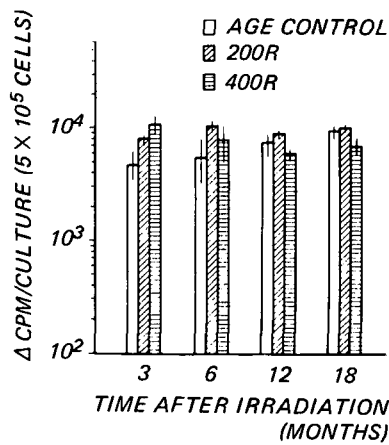


FIG. 5. LPS responsiveness of splenic lymphocytes of B6C3F₁/Nrs mice as a function of time after exposure to 200 R or 400 R X-rays.

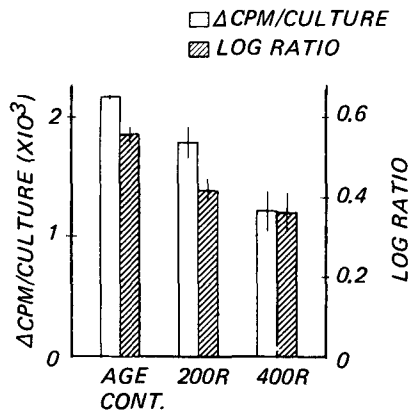


FIG. 6. Reactivity of splenic lymphocytes of B6C3F₁/Nrs mice to allogenic (B10.D2; H-2^d) lymphocytes in vitro 18 months after exposure to 200 R or 400 R X-rays. □ Δ counts/min per culture; ▨ log stimulation ratios.

significant ($0.001 < P < 0.01$) regardless of whether the statistical analysis was based on the net increase in the uptake of ³H-TdR (Δ counts/min) or on the log stimulation ratios. In contrast, the difference between the 200 R-treated and the control group was significant only when the statistical analysis was based on log stimulation ratios ($P < 0.001$).

4. DISCUSSION

Relatively few studies have been performed on the late effects of radiation on the immune system of mammals [18–23], and the results obtained from these studies were variable, depending upon the test systems and the radiation doses employed. In general, when intact animals were examined individually for any immunologic functions, few, if any, late effects were observed [18, 19, 23]. In contrast, when immunologic functions were tested with a fixed number of dispersed spleen cells obtained from irradiated animals, a significant degree of late effects was demonstrated [21–23].

In the present study, the immunologic competence of aging mice exposed to 150–450 R X-rays during their young adulthood was analysed using several different assay systems. All determinations were based on individual mice or individual spleens. The results indicated that, under the experimental conditions employed, we were not able to demonstrate a clear evidence of acceleration of aging of the immune system as a result of earlier exposure to radiation, apart

from the alloantigen reactivity of splenic lymphocytes derived from B6C3F₁/Nrs mice, which was shown to be suppressed when tested at 18 months following whole-body exposure to 400 R and, less significantly, to 200 R X-rays.

It may be added, however, that our more recent data obtained with C3Hf/HeMsNrs[SPF] male mice failed to show a depression of alloantigen reactivity of splenic lymphocytes when tested at 12 or 18 months following whole-body exposure to 143–572 R of ¹³⁷Cs gamma rays, although the sample size in this experiment was not sufficiently large to make a conclusive statement at this time. It is possible that the suppression of the alloantigen reactivity of splenic lymphocytes observed in B6C3F₁/Nrs mice exposed to 400 R X-rays 18 months earlier could be an artefact resulting from the suppression of the proliferative response of lymphocytes by contaminating non-lymphoid elements, particularly the macrophages [24–25]. The data shown in Fig.3 suggest strongly that this indeed could have happened in the spleens of the 400 R-treated mice. Thus the results shown in Fig.6 must be re-examined with special attention to this problem.

ACKNOWLEDGEMENT

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DISCUSSION

Y. NISHIWAKI: What do you think is the reason for the marked increase with dose in the number of total nucleated cells recovered from the spleens, as there is no significant difference in the number of T and B lymphocytes between the irradiated and non-irradiated age control groups?

T. SADO: This is a rather difficult question to answer at the moment, primarily because we do not have any information on the relative proportions of different cell types present in the irradiated as well as non-irradiated spleens. At the time of this experiment we were interested only in the counts of T and B lymphocytes. Now we realize that we need to know the total numbers of null lymphocytes, macrophages and myeloid as well as erythroid cells in addition to the counts of T and B lymphocytes. This kind of analysis is being included in the experiments now in progress using SPF and conventional mice on a comparative basis.

C. STREFFER: It is most surprising that you did not find a radiation effect on the parameters of the immune system which you investigated at later periods after irradiation. As you mentioned and as is well known, the immune

system is comparatively radiosensitive. Severe changes can be observed some days after radiation doses of 200–400 R, which is what you used in your experiments. Did you observe such acute effects in your experimental system?

T. SADO: It is, rather, true to say that the immune system is highly sensitive to radiation, and I pointed out this well-documented fact at the beginning of my presentation. One can easily demonstrate a dose-dependent suppression of many immunological parameters, if they are examined immediately after irradiation. We also have a great deal of quantitative data, not presented here, which show that all the immunological indices employed in this study are suppressed in a dose-dependent manner when tested shortly after radiation exposure. However, I deliberately confined myself to the late effects in this presentation.

DEVELOPMENT OF FIBROSIS IN DOGS AS A LATE CONSEQUENCE OF WHOLE-BODY X-IRRADIATION*

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Abstract

DEVELOPMENT OF FIBROSIS IN DOGS AS A LATE CONSEQUENCE OF WHOLE-BODY X-IRRADIATION.

Dogs were whole-body irradiated with a single mid-line dose of 1200 R at 300 kV. This high dose will kill all non-treated animals within a few days. To save these animals, leukocytes were previously collected during a four-hour leukapheresis using a continuous-flow centrifuge and were stored under liquid nitrogen. Shortly after the whole-body irradiation each dog received its own cryopreserved cells thawed immediately beforehand. The dogs received between 0.32×10^9 and 1.63×10^9 mononuclear blood cells per kilogram of body weight. The number of colony-forming cells contained in the transfusate ranged between 0.19×10^5 and 1.38×10^5 per kilogram of body weight. This blood stem transfusion, together with general supportive therapy, enabled the dogs to overcome the acute radiation syndrome and to recover. The dogs were subsequently sacrificed in two groups after observation for about 260 days or 700–898 days respectively. Pathological findings are described. A particular situation existed in the marrow, where non-irradiated stem cells had colonized bone cavities containing irradiated stroma. Progressive fibrosis developed in the endosteal areas of the bone cavities in most of the animals.

1. INTRODUCTION

Fibrosis has been described as a non-neoplastic late effect in a number of tissues after exposure to ionizing radiation, and its pathogenesis remains a subject of scientific interest and investigation.

In the course of a preclinical study on the possibilities and limitations of treating the acute radiation syndrome as a consequence of a 'supralethal' (1200 R) whole-body X-irradiation by means of blood stem-cell transfusions [1, 2], we had

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the opportunity to follow seven dogs for several hundred days and to study the various tissues at the time of death. Since these dogs had received a transfusion of their own blood mononuclear leukocytes removed prior to irradiation by continuous flow leukapheresis and kept frozen until transfusion [3], the radiation late effects observed were not associated with immunological reactions, but rather should be regarded as direct consequences of the whole-body X-irradiation.

It is therefore the purpose of this report to present evidence for the development of fibrotic lesions in various organs of seven dogs that survived a 1200 R whole-body X-irradiation. This report may be of immediate clinical interest since whole-body irradiation with doses of about 1000 R is being employed in a number of clinical centres to condition human beings preparatory to bone marrow grafting to treat aplastic anaemia or leukaemia. In such clinical trials, the possible development of non-neoplastic radiation late effects should be taken into account before the decision to perform autologous or allogeneic stem-cell transplantation is made.

2. MATERIALS AND METHODS

2.1. Animals

Fifteen adult male and female dogs (beagles) of the same colony were used in this study. The animals were vaccinated against hepatitis and distemper, and dewormed several months before the onset of the experiment. Eight dogs were irradiated and transfused with their own cryopreserved blood leukocytes. Seven dogs were not treated, and served as controls.

2.2. Radiation exposure

The dogs were whole-body X-irradiated with a midline dose of 1200 R delivered by a Siemens Stabilipan X-ray machine at a dose rate of 7.2 R/min (in the midline of a phantom), using a Thoraeus III filter and an HVL of 5 mm Cu. The distance from the focus to the proximal surface was 120 cm. Half of the dose was given to one side and the remainder to the other side of the animal.

2.3. Collection, storage and transfusion of leukocytes

Leukocytes were collected from the dogs during a four-hour leukapheresis with an NIH-IBM continuous-flow blood cell separator connected to an arterio-venous shunt. The leukocytes were frozen in the presence of 10% dimethyl sulphoxide (DMSO) in a Cryoson Freezer and stored for two months in liquid nitrogen. Within hours after irradiation the dogs were given intravenously their own cryopreserved cells, thawed shortly beforehand. Details of this procedure

and the treatment and care of the animals after irradiation have been published by Fliedner and co-workers [2, 3]. The dogs received between 0.32×10^9 and 1.63×10^9 mononuclear blood cells (MNBC) per kilogram body weight.

2.4. Haematology studies

A sample of the suspension of cells to be transfused was cultured in agar according to the method of Bradley and Metcalf and adapted to our experimental conditions by Kovács and co-workers [4]. The number of colony-forming cells (CFUc) calculated to be present in the transfusate was found to range between 0.99×10^5 and 1.38×10^5 per kg body weight for the first group of dogs ($n = 4$), and between 0.19×10^5 and 0.43×10^5 body weight for the dogs of the second group ($n = 3$). One dog received only 0.02×10^5 CFUc per kg body weight.

The dogs were examined haematologically at regular intervals before and for several hundred days after irradiation and blood leukocyte transfusion. Details of the methods used, as well as the general findings, have been or will be reported elsewhere [2].

2.5. Pathology studies

The irradiated and stem-cell-treated dogs were divided into two groups for sacrifice. The reason for sacrifice was scientific; the dogs were in good health at this time. One dog, however, showed signs of impairment of movement a few days before sacrifice. Group 1 included four dogs observed for 700 to 898 days. Group 2 consisted of three dogs that had been observed for 259 to 261 days. A fourth dog, originally in group 2, died 20 days after irradiation and transfusion of an insufficient number of stem cells. No late effects could, therefore, be observed and this dog was excluded from the late effect study (Table I). A third group contained seven healthy control dogs. Three of them were of the same age as the longest surviving animals. The other four were younger. A complete autopsy was performed, taking samples of every organ as well as fragments of ribs, humerus, radius and tibia from all irradiated and control dogs.

3. RESULTS

3.1. General course

The seven dogs that had received 1200 R whole-body X-irradiation followed by a transfusion of autologous, cryopreserved mononuclear leukocytes (containing between 0.19×10^5 and 1.38×10^5 CFUc per kg body weight) all showed a

TABLE I. LATE SOMATIC EFFECTS OF 1200 R WHOLE-BODY X-IRRADIATION IN DOGS TREATED BY TRANSFUSION OF 0.3 to $1.6 \times 10^9/\text{kg}$ BODY WEIGHT AUTOLOGOUS BLOOD LEUKOCYTES

Dog No. ^a Organ	1 (701)	2 (845)	3 (743)	4 (898)	5 (259)	6 (259)	8 (261)
<i>Lung:</i>							
Fibrosis	+	0	0	0	0	+	+
Bronch. papilloma	+	0	0	0	0	0	0
Infarct	0	+	0	0	0	0	0
<i>Spleen:</i>							
Art. hyaliniz.	+	++	+	++	+	+	++
Infarct	0	+	0	0	0	0	0
<i>Kidney:</i>							
Glom. sclerosis	+	+	++	+++	+	+	+
Tubular degen.	0	+	+	+++	+	+	++
<i>Gonads:</i>							
Aplasia	+++	++	++	+++	++	++	+++
<i>Marrow:</i>							
Endosteal fibrosis	+	+	0	+	++	+	+
<i>Pancreas:</i>							
Fibrosis	0	0	+	0	+	+++	0
Adenoma	0	+	0	0	0	0	0

<div>Dog No.^a</div> <div>Organ</div>	1 (701)	2 (845)	3 (743)	4 (898)	5 (259)	6 (259)	8 (261)
<i>Other findings:</i>	Coxarthros.	Dural fibr. <i>Liver:</i> Atrophy +	0	<i>Brain:</i> Gliosis + Necrosis +	0	Heart infarct	<i>Liver:</i> Sclerosis + <i>Pituitary:</i> Cyst

^a (survival in days) Dog 7 not included in the table because of short survival; 0 = no lesion, +, ++, +++ = minimal, moderate, severe changes.



FIG.1. The fibrotic connective tissue attached to the bone trabeculae reduces the space available for the marrow. Dog 5, sternum. (H and E, $\times 200$)

'take', in the sense that their bone marrow, and subsequently the entire haematopoietic cell renewal system, recovered, as reported elsewhere [2, 3, 5]. The bone marrow cellularity and the blood counts returned to normal levels within 100 days. This blood stem-cell transfusion, together with the general replacement therapy (electrolytes, antibiotics, platelet transfusions) allowed the dogs to overcome the acute radiation syndrome and to recover. After overcoming the consequences of the acute radiation syndrome, the dogs appeared healthy, except that their fur lost its normal colour and became prematurely grey.

3.2. Haematological observations before sacrifice

At the end of the observation period, most of the dogs had blood counts similar to those before irradiation, except for one dog that showed a slight anaemia. The differential counts in the marrow smears shortly before sacrifice showed that the proportion of granulopoietic to erythropoietic cells was close to normal. The relative concentration of lymphocytes, on the other hand, was reduced to about 40% of normal in the dogs of group 1, and to a less marked degree in the dogs of group 2, observed for a shorter time than those of group 1.

3.3. Pathological observations at the time of sacrifice

The major pathological findings in the seven dogs that had survived the 1200 R whole-body X-irradiation for more than 100 days are given in Table I. At the time of sacrifice, fibrotic tissue was observed in the marrow cavity of the bones in six out of seven irradiated dogs. The fibrosis was slight in most of the dogs, but in one dog, sacrificed 259 days after irradiation, the fibrosis was advanced to the point of obliterating some of the osteal niches formed by the bone trabeculae. In the same bone, one could observe areas free of fibrotic tissue as well as various stages of invasion of osteal niches by connective tissue, as demonstrated in Fig. 1. The connective tissue was dense, contained some mast cells and was attached to the bone trabeculae, at which location there were no osteoblasts. None of the control dogs showed such a fibrosis in the bone cavities.

In the lungs, fibrosis was present in three dogs, but was not observed in three of the dogs surviving more than 700 days. A small bronchial papilloma was observed in one dog, and another dog showed evidence of an old infarct.

There was evidence of arterial hyalinization in the spleen of all irradiated dogs to a greater or lesser degree. In the control dogs, such a hyalinization was occasionally present, but was always much more extensive in the irradiated animals. In one dog, an infarct lesion was seen.

The kidneys of all irradiated animals showed glomerular sclerosis. In dogs Nos 3 and 4, these changes were extensive. In control dogs, one might expect some degree of glomerular sclerosis but, again, the changes were much more

pronounced in the irradiated dogs. Tubular degeneration was extensive in six of the seven irradiated dogs, and of particularly great extent in the longest survivor (898 days — almost 2 1/2 years).

Fibrotic lesions were also noted in the pancreas in four of the seven dogs. In dog No. 6 the lesions were quite extensive. An adenoma was seen in one animal. Such lesions were not observed in any of the unirradiated control dogs studied.

Finally, the gonads of these dogs (five males and two females) showed extensive aplasia. However, it was of interest that, in a separate experiment using allogeneic blood stem-cell transfusion after 1200 R whole-body X-irradiation, one irradiated female recipient dog became pregnant.

Other findings, such as gliosis of the brain or liver changes, are listed in Table I.

4. DISCUSSION

A midline dose of 1200 R is well above the LD 50/30 for dogs. The intravenous transfusion of autologous cryopreserved mononuclear leukocytes was followed by repopulation of the marrow, allowing the animals to survive by avoiding the deadly syndrome of marrow aplasia that appears as a consequence of high-dose, whole-body irradiation. Immunologic reactions, such as graft-versus-host, were also avoided by the transfusion of autologous cells removed from the blood before irradiation. In the present system, there is marrow formed by a parenchyma originating from non-irradiated stem cells and an irradiated stroma. The question whether the irradiated stroma, receiving at least 1200 R, would be able to support, for a long period of time, a practically normal haemopoiesis has been answered affirmatively by the present experiment. The presence of a progressive fibrosis, occupying an increasing amount of space in the marrow cavity, may cause impairment of haemopoiesis after longer periods of time. The reduction of the space available to the marrow may, for a while, be compensated for by extramedullary haemopoiesis, located mainly in the spleen.

The fibrosis found in the spongy bones of our animals, in coexistence with a morphologically and functionally normal marrow, developed in the absence of immunologic reactions, and is different from the myelofibrosis observed by Stodtmeister and Fliedner [6] after transfusion of irradiated rats with allogeneic bone marrow cells. The fibrosis described by these authors starts with necrobiotic changes of the haemopoietic cells; connective tissue is found interspersed among the blood-forming cells. These changes were interpreted as being due to an immunological interaction between the irradiated matrix of the marrow and the engrafted haemopoietic cells. In our dogs, the fibrotic tissue attached itself to the bone trabeculae and progressed toward the space occupied by the marrow.

The haemopoietic tissue kept a normal appearance. The fibrosis observed in our animals has not been described before, to the best of our knowledge, and appears to be a 'late consequence' of the irradiation. For this type of lesion we propose the term of 'endosteal fibrosis'. The pathogenetic mechanisms for the development of this type of 'endosteal fibrosis' and the role of the particular quality of radiation used (300 kVp X-rays) need to be explored. As shown in Table I, the non-haemotopoietic organs of most of the irradiated animals developed non-neoplastic lesions that, in principle, are known to develop as a late consequence of radiation exposure, such as after local irradiation in man for therapeutic reasons. It is of interest that some of these lesions were also seen in non-irradiated control dogs of the same age, but to a markedly less extent (such as glomerular sclerosis). In these cases, it appears that the non-neoplastic radiation late effects are not 'radiation-specific', but are rather reactions of the organs that can occur as a result of other aetiological factors. Here, the radiation appears to play the role of an 'amplifier' or of an 'enhancer'. In other instances (pancreas fibrosis), lesions were found only in irradiated animals. However, in all these instances it must be concluded that the late-effect lesions develop on the basis of 'ineffective' reactivity of the supporting, connective tissues. This indicates that in these tissues a 'restitutio ad integrum' is not possible after exposure to ionizing radiation, and that residual damage must be recognized and quantified before a prognostic evaluation can be performed.

This report is dedicated to Professor R. Stodtmeister, on the occasion of his 70th birthday, as a pioneer in the understanding of the pathogenesis of myelofibrosis.

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DISCUSSION

V.M. VOLODIN: Could you say something about the role of possible damage to the microcirculation system in the development of fibrosis in bone marrow after whole-body X-irradiation? Have you any data on this subject?

W. CALVO: The lesions of the vessels in the marrow were not as marked as in other organs such as the kidney and spleen. We found in the marrow a slight reduction in the number of endothelial cells of the sinuses, this being more pronounced in the animals killed after a longer period of time. The missing cells may have died as a consequence of the lesion received during the irradiation, manifested only when it became necessary for them to divide in order to replace endothelial cells that had reached the end of their normal life span. Examination of the marrow sections indicated that most of its microcirculation was intact. We are inclined, therefore, to interpret the development of the endosteal fibrosis as a consequence of a metaplasia of the osteoblastic layer, whereby dense fibrotic tissue was produced instead of bone, and to consider the metaplasia as a late effect of the irradiation manifested in the endosteal area.

HISTOLOGICAL AND STEREOLOGICAL ANALYSIS OF SOME ENDOCRINE AND LYMPHATIC ORGANS OF MICE AFTER WHOLE-BODY IRRADIATION*

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Abstract

HISTOLOGICAL AND STEREOLOGICAL ANALYSIS OF SOME ENDOCRINE AND LYMPHATIC ORGANS OF MICE AFTER WHOLE-BODY IRRADIATION.

This investigation was carried out on 24 mice of the CBA strain with a mean age of 1.5 months. They were distributed into three groups and matched as to sex, age and body weight. When 2.5 months old, group 1 was sacrificed, and group 2 was subjected to whole-body gamma irradiation with 20 successive daily doses of 50 rads, the total dosage applied being 1000 rads; group 3 consisted of non-irradiated controls. The following organs were selected for histological and systematic stereological analyses: adrenal, thyroid gland, ovary, testis and thymus. The animals of groups 2 and 3 were sacrificed at the age of 8.5 months (i.e. six months after the beginning of irradiation). The method of variance analysis was utilized to evaluate the effects of age and irradiation, and interaction between the two.

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Compared with the non-irradiated controls, the irradiated mice showed the following significant differences: the adrenal cortex was thicker and smoother outside, the fascicular zone rate augmented on account of other zones, vacuolization of the cytoplasm in it being considerably poorer. The thyroid follicles were bigger, more wrinkled and had a thinner epithelium. The ovaries were smaller and smoother, the functional parenchyma rate was smaller, all oocytes and follicles including the atretic ones were missing. The yellow bodies were changed, the interstitial cells being hypertrophic and hyperplastic. The inner surface of the seminiferous epithelium was slightly rougher, but testes showed no other significant alterations. The thymus was smaller in most cases, and revealed a less clearly delineated cortex and medulla. Volume, surface and vascularization of the medulla were reduced. A lymphoblastic lymphoma observed in one case was most likely a response to the ionizing radiation noxa. There was a broad spectrum of different late effects entailed by the sublethal fractionated whole-body irradiation on different organs. In some of them the regeneration and redifferentiation which followed the initial destruction were not complete. Some alterations could have resulted from post-irradiation stress.

INTRODUCTION

The present study is a part of an extensive project undertaken to determine the role played by neuro-endocrine factors in oncogenesis [1, 2]. The psychosomatic stress was elicited by an optic signal immediately followed by a slight electric stroke on the ground of the cage, where the animals were housed. This combined stress mechanism was repeated four times hourly for six hours daily and five days per week. Some results have been already reported [3–8], and more will be presented in the future.

This report is restricted to the description of the late effects of the sublethal whole-body fractionated irradiation observed in some endocrine and lymphatic organs.

MATERIALS AND METHODS

Twenty-four mice of the CBA strain of both sexes, six weeks old, were distributed into three groups of eight animals each. The mice were matched as to sex, age and body weight. In each group, half were exposed to the stressing conditions described above, and the other half, i.e. the controls, were not stressed. Mice were subject to stress throughout the study, from the beginning until they were sacrificed.

Group 1 was sacrificed one month after the beginning of the investigation. Group 2 was then submitted to whole-body irradiation carried out with a ^{60}Co therapy unit with 20 successive daily doses of 50 rads, the total dosage amounting to 1000 rads. Group 3 consisted of non-irradiated controls. Animals of groups 2 and 3 were sacrificed six months after the beginning of irradiation.

Mice were irradiated in hollow plexiglass wheels containing 16 separate boxes, 2.7 cm high and 7 cm long. As the cages and the irradiation place were quite far apart, animals were kept enclosed in their boxes for about one hour daily. All the animals of groups 2 and 3 were subjected to the above confinement stress. After sacrifice of the animals under ether anaesthesia, the adrenal, thyroid gland, ovary, testis and thymus were removed, fixed in Bouin's fluid and embedded in paraffin. Step serial sections of about 6 μm were stained with haematoxylin-eosin and studied by light microscopy at objective magnifications ranging from $\times 10$ to $\times 63$. Using systematic stereological analysis we estimated the volume densities and absolute volumes, surface densities and absolute surfaces, length densities and absolute lengths, numerical densities and absolute numbers of the particular tissue and cell components of the organs studied. Taking into account the geometry of the investigated organs, some derived variables were calculated from the original ones, e.g. the mean thickness of a particular layer [9].

Computer statistical analysis of the results obtained using the method of variance analysis was employed to evaluate the effects of age and irradiation, as well as the interaction of both. This study does not take into consideration the influence entailed by the stimulation, i.e. the psychosomatic stress.

RESULTS

As a rule, only those results showing statistically significant differences between the independent signs of the irradiated and non-irradiated animals ($P < 0.05$) are reported.

Adrenal

The adrenals of the irradiated mice showed less distinctly delineated cortical zones. The external surface density was diminished ($P < 0.0001$) and also the absolute external surface of the gland ($P < 0.0001$). The mean thickness of the cortex was increased ($P < 0.0008$). There was a simultaneous increase in the volume density of the fascicular zone ($P < 0.0001$) because of the diminished volume densities of the blastemic zone ($P < 0.004$) and glomerular zone ($P < 0.003$, Fig. 1). The volume density of the vacuoles in the fascicular zone cells was diminished to one fifth of the control value ($P < 0.03$).

Thyroid

The thyroid glands of the animals exposed to irradiation had bigger follicles showing a less regular shape and a more wrinkled surface. Desquamation of the epithelium into the colloid was observed in some regions, as well as the confluence of some follicles, some of which contained a poorly stained colloid.

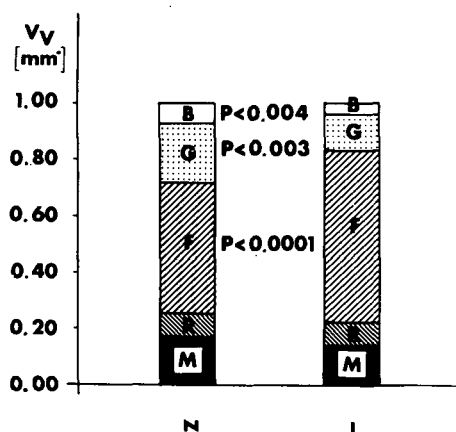


FIG. 1. Volume densities of the blastemic (B), glomerular (G), fascicular (F), reticular or X xone (R) and medulla (M) of the non-irradiated (N) and irradiated (I) mice; with the statistically significant differences the risk levels (P) are denoted.

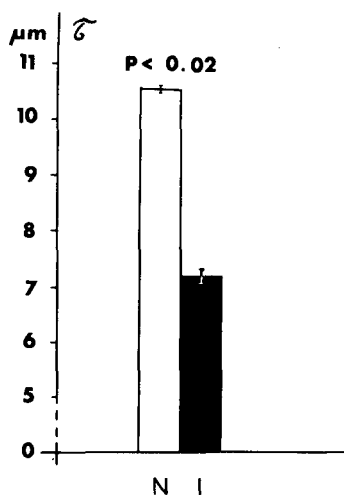


FIG. 2. Mean height of the thyroid epithelium (\bar{T}) of the non-irradiated (N) and irradiated (I) mice (Ref. [7]).

The external and internal surface densities of the follicles were increased ($P < 0.05$), but the mean thickness of the irradiated animals' epithelium was decreased ($7.1 \mu\text{m}$) compared with the epithelium of the animals not exposed to radiation ($10.5 \mu\text{m}$, $P < 0.02$, Fig. 2).

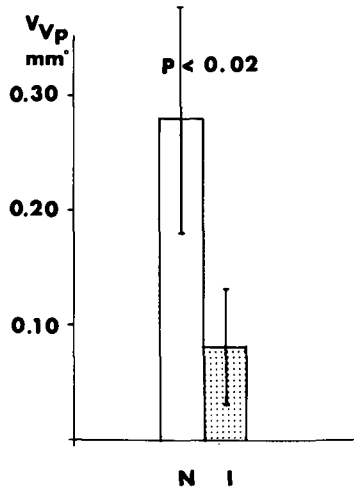


FIG.3. Volume density of the ovarian functional parenchyma (V_{VP}) of the non-irradiated (N) and irradiated (I) mice (Ref. [8]).

Ovaries

In both the irradiated and non-irradiated females all phases of the sex cycle were present, with the exception of the oestrus. The ovaries of mice exposed to irradiation were depleted of all oocytes as well as primary, secondary, tertiary and atretic follicles. The cortex contained a number of epithelial rings with no oocytes, and in the medulla hyperplastic and hypertrophic interstitial cells were found. The cytoplasm of sparse yellow body cells contained homogeneous eosinophile droplets. The ovaries of the irradiated animals had a smaller volume ($P < 0.02$), smaller absolute surface and specific surface density ($P < 0.003$), and lesser volume density of the functional parenchyma ($P < 0.02$, Fig.3) compared with the ovaries of the non-irradiated mice. The volume density of the interstitial cells increased. The aging process was observed to have brought about a decrease in number and total volume of the follicles and yellow bodies as well as the atretic follicles rate.

Testes

In the testes of both the exposed and non-exposed mice, sperm-agglutinates were observed. The epithelium of the irradiated animals had fissures spreading radially from the lumen towards the basal membrane, which increased the inner surface density of the seminiferous tubules ($P < 0.03$). No other significant differences between the irradiated and non-irradiated males were observed.

Thymus

There were differing responses of the thymus to irradiation. In one case a lymphoblastic thymoma developed. Some animals showed only scarce, hardly recognizable remnants of the thymus but mostly there was no clear limit between the cortex and medulla; in the latter numerous cysts were found. Some irradiated mice had normal structure of the thymus according to age. Stereological analysis showed a decrease in medullar volume ($P < 0.008$) and surface densities ($P < 0.01$) as well as length density of the medullar vessels ($P < 0.006$). There was an evident age involution of the cortex, medulla and stroma.

DISCUSSION

Our findings show a considerable variety of late effects exerted by the sublethal fractionated whole-body irradiation of the endocrine and lymphatic organs under study. Only a partial comparison between our results and the available data from the literature is possible because of the difference in doses applied and the difference in the conditions under which the irradiations and observations were performed. In contrast with most other histological data available, our results have been quantified.

Adrenal

In the literature the adrenal is reported to be highly radioresistant [10–12]. The therapeutic doses show hardly any histological effect [10]. Biochemical studies performed on rats [13, 14] and guinea pigs [11] suggested an increased activity of the adrenals in the first days after irradiation. This is in accordance with the histologically proved hypertrophy of the fascicular zone and depletion of the cortical lipids following the exposure of the rat's abdomen, neck or head to 2800 rads irradiation [11]. Cellular degeneration of the medulla and its atrophy resulting from the maximum irradiation dose have been reported [10]. Half a year after irradiation we observed signs evidencing an intensified activation of the fascicular zone; decreased vacuolization, however, indicated that it was exhausted. A smaller blastemic zone suggested a reduced regeneration capacity of this gland. These alterations could have been caused by the post-irradiation stress, probably in the phase of exhaustion.

Thyroid

Our findings concerning the thyroid gland agree with previous observations, which report a functional depression of the gland due to the sublethal X-ray

doses bringing about an augmentation of the follicles and thinning of the epithelium [15]. However, there are still conflicting views as regards the mechanism of the effect. According to some authors, the decisive part of the depression of TSH secretion is based on the prevailing ACTH production; they deny a direct effect upon this gland [16]. Our findings are in agreement with this argument. Some authors tolerate a direct effect of irradiation, but consider the hypothalamic paraventricular nucleus [17], as well as the epiphysis alone, or the latter together with the epithalamic region [18] to be involved too. At present, our data on the organs of the central neuro-endocrine regulation are not available.

Ovaries

Our studies of the ovaries up to the present show that different kinds of stress can cause an unspecific hindering of the ovulation and acceleration of the atresia [2, 5, 19]. The ovarian response to irradiation, however, is of a highly specific nature [8]. The ionizing radiation destroyed all the oocytes, which were mostly in the prophase of the first meiotic division. The available literature data report a very high radiosensitivity of the mice oocytes [20]. All parenchymal components were reduced, except the interstitial cells and yellow bodies, in which the signs of damage were also present. The interstitial cells are generally considered as highly radioresistant according to Lacassagne (quoted in Ref.[20]); they originate from the theca interna of the atretic follicles and are ascribed the role of oestrogen secretion. The interstitial cells and yellow bodies of the irradiated mice are very likely to have preserved their hormonal activity, since the histological appearance of the other parts of the sex organs remained the same in both the irradiated and control animals. These findings agree with those reported in the literature, which point out that the secretion of oestrogens does not cease after the exposure to irradiation [20].

Testes

In contrast with the marked post-irradiation alterations involving the ovaries, scarcely any changes were observed in the testes. Sperm agglutination occurred in the testes of mice exposed to various kinds of stress or other noxious effects [4, 21, 22]. Generally, one month after irradiation a complete regeneration of the testis was reported [11, 23]. With the mouse 5050 rads is considered as a sterilization dose [11]. A complete sterilization of the testes is hindered by the fact that different spermatogonia exist at the same time, being either in the phase of radiosensitivity or radioresistance [24, 25]. Consequently, it is not surprising that even the most meticulous stereological analysis revealed only increased internal surface density of the seminiferous epithelium, which could account for its higher vulnerability [26].

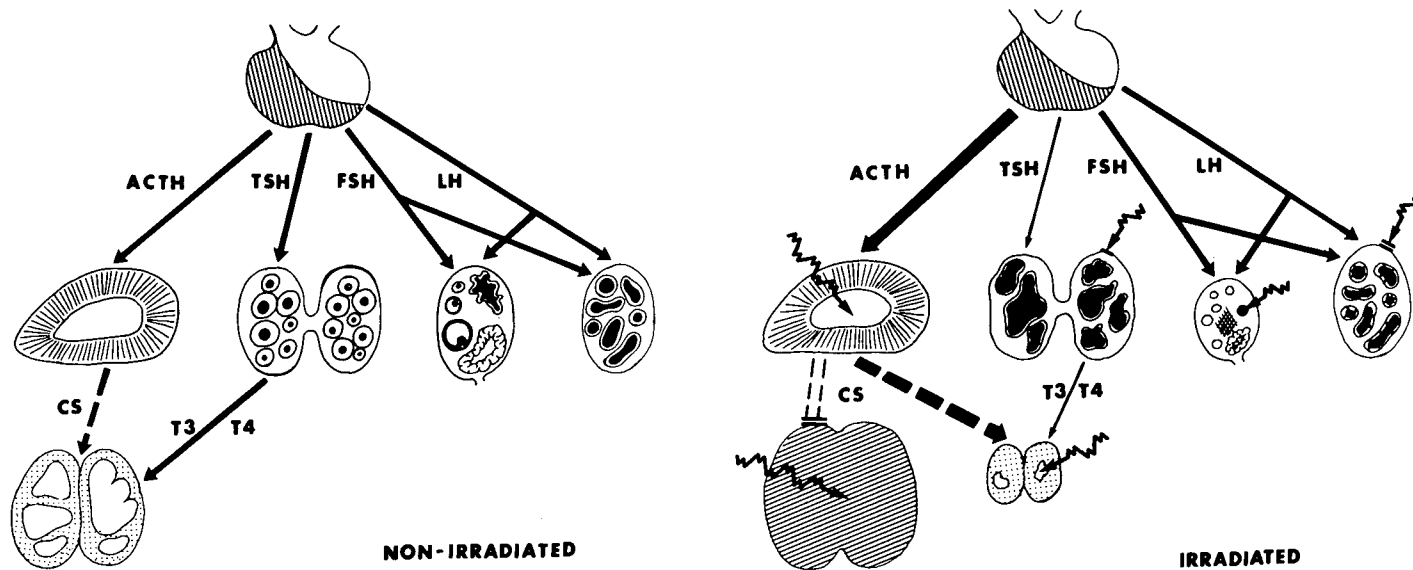


FIG. 4. Hypothetical interpretation of the response of some endocrine and lymphatic organs to irradiation:

D = destruction

R = regeneration

U = incomplete differentiation

M = malignant alteration

S = stress

Adrenal: DRUS

Thyroid gland: (D)RS

Ovary: DRU

Testis: (D)R

Thymus: DRUMS

Thymus

Our observation that the effect of the age involution on the medulla of the thymus is disproportionally greater than its influence on the cortex is not in agreement with the available literature reports [27, 28]. As other authors either weighed the thymus, or tried to determine the qualitative or quantitative signs differing from those estimated by our study, their results can hardly be compared with ours. Besides, the time elapsed from irradiation until their investigation of the thymus was shorter (three months at the most). Also, there was a difference in irradiation doses and other irradiation conditions [29, 30–32]. An increased post-irradiation involution of the thymus medulla assessed could have resulted partly from an incomplete regeneration and redifferentiation following the initial destruction after ionizing radiation, and partly from the disturbed hormonal balance in the organism (Fig.4). ACTH hypersecretion might have given rise to a faster involution through a higher corticosteroid production rate. On the other hand, however, reduced TSH stimulation of the thyroid gland could have entailed an additional negative influence on the size of the thymus due to decreased thyroid hormone stimulation. The somatotrophic function of the adenohypophysis cannot be discussed until histological data on the pituitary gland have been provided.

CONCLUSIONS

In accordance with the above observations we can conclude that in all organs tissue destruction to a lesser or greater extent occurs as a result of the direct damage of the ionizing radiation to the cells. In all cases observed this destruction was followed by a more or less efficient regeneration process (except in the ovaries, which were sterilized). The regenerated tissue of some organs, however, showed evidence of an incomplete redifferentiation. The damage seemed to be too severe for some organs to compensate for it. Moreover, in some organs, i.e. adrenal, thyroid gland and thymus, some structural changes could be regarded as the consequences of the post-irradiation stress. Finally, the thymoma might have developed as a response of the thymus to the ionizing radiation noxa.

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HIGH LET IRRADIATION OF SPRAGUE-DAWLEY FEMALE RATS AND MAMMARY NEOPLASM INDUCTION

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Abstract

HIGH LET IRRADIATION OF SPRAGUE-DAWLEY FEMALE RATS AND MAMMARY NEOPLASM INDUCTION.

It has been demonstrated that low doses of neutrons will induce a significant number of mammary neoplasms in rats. It seems important to determine whether other ions of high LET (probably to be used for human therapy) will also have a high RBE, especially at low doses. Therefore, the object of this experiment was to study the effects of high-energy neon ions in the induction of mammary carcinogenesis in young, adult, female, Sprague-Dawley rats. Two hundred and five rats (Sprague-Dawley, Simonsen) were exposed at the BEVALAC facility to neon doses of 2, 20, 50, 100, 150, and 200 rads. There were 49 unirradiated controls. Four mammary tumours were induced eight months after the highest three doses. No mammary neoplasms were induced in the range 2–50 rads. Shellabarger, in a similar experiment, did find some tumours after low doses. In 1976 Baum proposed a model which postulated that cancer induction was a multi-event process. This model was tested by comparing single and split doses of neutrons (with 24-hour intervals between equal fractions). There was no significant difference between the single (10, 20, and 70 rads) doses and the paired neutron doses in the induction of mammary tumours in these Sprague-Dawley rats. The neutron/X-ray RBE values were compared for the induction of mammary neoplasms in Sprague-Dawley rats (Montour (mean 14.5 MeV neutrons), Shellabarger (0.43 MeV), Broerse (15 MeV and 0.5 MeV) and Vogel (mean fission spectrum of 1.2 MeV)). The RBE value increases as the neutron dose decreases. The RBE may reach 50 to 100 at low neutron doses. In contrast, comparing high neutron doses, the RBE values were found to lie between 4 and 5. The importance of these data as applied to human radiation problems, such as leukaemia incidence in Hiroshima and Nagasaki and the mammo-graphy problem, are discussed. The data indicate that the neutron/X-ray RBE values should be carefully studied in several biological systems and, perhaps, an elevated neutron 'quality factor' be recommended for health physicists' use.

INTRODUCTION

The BEVALAC, a versatile, high-energy, heavy-ion accelerator complex, has been in operation at the Lawrence Berkeley Laboratory of the University of California for less than three years [1]. The first successful acceleration of heavy

ions to high energies at Princeton and Berkeley generated interest and excitement in not only the biomedical staff of Lawrence Berkeley Laboratory, but in many other laboratories among the more than two hundred users of the facilities. Heavy-ion beams have been useful for some time in nuclear chemistry, particularly for creation of transuranium elements and the study of nuclear excited states.

A major purpose for which this accelerator complex was constructed was to explore the possibility of heavy-ion beams for therapy for certain forms of cancer in man. Significant progress has been made in this direction. Heavy ions have now been included by the National Cancer Institute (United States of America) in a long-term plan for particle therapy that will assess by means of controlled therapeutic tests the value of various modalities. A new diagnostic method known as heavy-ion radiography has shown greatly increased sensitivity for soft-tissue detail [1]. It may become a powerful tool for localizing early tumours and metastases. The BEVALAC will also be useful for the continuation of previously developed methods for the control of acromegaly, Cushing's disease, and, on a research basis, advanced diabetes mellitus with vascular disease. The ability to make small, bloodless lesions in the brain and elsewhere with heavy-ion beams has great potential for nervous system studies and perhaps later for radioneurosurgery.

With regard to the main study of heavy ions in relation to cancer therapy, rapid progress is being made to understand and control the physical interactions of the beam. Irradiation with heavy ions provides superior ability to deliver advantageous depth-dose patterns which are much superior to those that can be produced by exponentially absorbed beams, such as gamma rays or neutrons. The versatility of heavy-ion acceleration lies in the fact that it will be possible to deliver optimal beams for a variety of purposes: argon and neon beams for shallow tumours requiring low-oxygen effect; neon beams for deep-seated tumours; carbon and helium beams when the body region affected by cancer is very large; carbon beams for providing small, well-localized radiolesions.

A special area at the BEVATRON has been designated as the BEVALAC Biomedical Facility [2]. Within this area three irradiation rooms are available and instrumentation in an appropriate manner to allow the controlled irradiation of selected biological objects.

1. INDUCTION OF MAMMARY NEOPLASMS IN FEMALE SPRAGUE-DAWLEY RATS BY IRRADIATION WITH HIGH-ENERGY NEON IONS

1.1. Experimental aims

The object of this experiment was to study the effects of high-energy ions from the BEVALAC, Berkeley, California, in the induction of mammary

carcinogenesis in young, adult, female Sprague-Dawley rats. We have demonstrated [3-5], and others have confirmed [6], that very low doses of fission neutrons (1 to 2 rads) will induce a significant number of mammary neoplasms in this rat. We believe it important to determine whether other ions of high LET radiation will also have a high RBE, especially at low doses, for these biological effects. Since, in the long run, some of these ions will be used for human therapy, it seems imperative that we answer this problem in radiation-induced carcinogenesis. Other experiments have already been carried out with low LET, X-, and gamma rays [7]; with fission neutrons from reactors [3] and from ^{252}Cf [8]; and with Van de Graaff neutrons of lower energy [5]. We have carried out two experiments at BEVALAC with neon ions (total of 6 h of irradiation time). In the first run (A) on 6 March 1977:

50 rats were exposed to 2 rads Ne
40 rats were exposed to 20 rads Ne
30 rats were exposed to 50 rads Ne.
(There were 34 unirradiated controls.)

In Experiment A, two rats were irradiated simultaneously. In Experiment B, single rats were exposed. The second run (B) was carried out on 26 July 1977:

30 rats were exposed to 100 rads Ne
30 rats were exposed to 150 rads Ne
25 rats were exposed to 200 rads Ne.
(There were 15 unirradiated controls.)

The dose rate was varied so that the total mean dose was delivered in approximately one minute.

1.2. Animals and facilities

The Sprague-Dawley rats were purchased for us by the Lawrence Berkeley Laboratory and retained at their animal facilities for several weeks before irradiation. The female, albino rats were two months old when irradiated. They came from Simonsen Laboratories, Gilroy, California, United States of America. After exposure, the animals were retained at Berkeley for about a week and then were shipped by air to Memphis. The rats were placed, five to a cage, on racks in our Animal Resource Division's animal facilities at the University of Tennessee Center for the Health Sciences on the ground floor of the Walter Chandler Clinical

MAMMARY TUMOURS INDUCED BY
X-RAYS, FISSION NEUTRONS AND NEON IONS
(300 DAYS AFTER EXPOSURE)

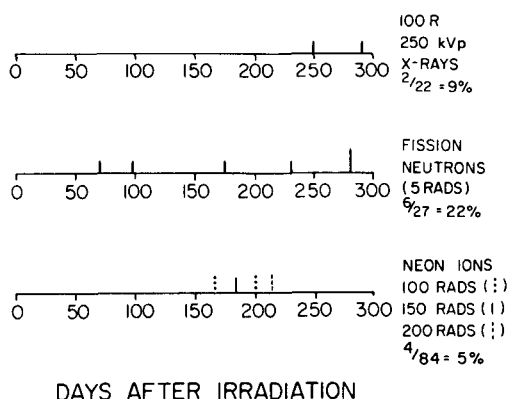


FIG.1. Mammary neoplasms induced by 100 R of X-rays, 5 rads of fission neutrons, and 100–200 rads of high-energy neon ions up to 300 days after exposure.

Services Center. The animals were ear-punched for positive identification. The temperature was controlled at $24 \pm 2^\circ\text{C}$. Illumination in the animal quarters was 12 h on, 12 h off.

1.3. Results

Among the 120 neon-irradiated rats in Experiment A (2–50 rads) and the 34 unirradiated controls, there were no mammary tumours palpated up to one year after exposure (6 March 1978) when the experiment was terminated.

Since 50 rads of fission neutrons produced mammary tumours in 26/31 (84%) irradiated Sprague-Dawley rats within 400 days of exposure, we conclude that fission neutrons are much more efficient as physical carcinogenic agents for mammary tumour induction in this rat than are high-energy neon ions. The dose median LET was estimated to be about $50 \text{ keV}/\mu\text{m}$ for small animal geometries for the DOSAR Reactor neutron spectrum at Oak Ridge National Laboratory [9].

In the second experiment in which exposures of 100, 150 and 200 rads of neon were used, we have found four mammary tumours eight months after irradiation. We expect that more will be found as time goes on, judging from our other irradiation experiments in inducing mammary carcinogenesis.

In Fig.1, we have illustrated the latent period to the induction of these four mammary tumours induced by neon ions. The percentage of rats with mammary tumours is still very low (3–6%) in this group of rats irradiated with 100–200 rads of neon. There were no mammary tumours in the 49 unirradiated controls

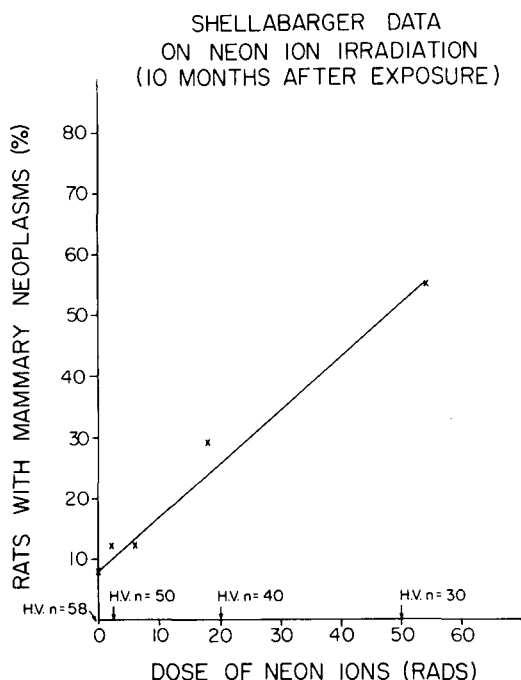


FIG.2. Mammary neoplasms induced by 2–54 rads of high-energy neon ions (BEVALAC), 10 months after exposure. (Data of C.J. Shellabarger.)

(Experiments A and B). In this figure, we have compared neon-irradiated animals with other rats exposed to 5 rads of fission neutrons and to 100 R of 250 kVp X-rays. Each vertical line represents one mammary neoplasm. It is clear that 5 rads of fission neutrons produce more mammary tumours than 200 rads of neon ions in a 300-day period after exposure. It is also of interest that 100 R of X-rays and 100 rads of neon do not appear to be significantly different over this time period as physical carcinogens for mammary tumour induction in the Sprague-Dawley rat. The RBE should be close to unity.

During 1977 at the Berkeley BEVALAC, two new investigations of radiation carcinogenesis, unrelated to space research, have been started (Ref. [1], p. 17). C.J. Shellabarger, of Brookhaven National Laboratory, and H.H. Vogel, Jr., of the University of Tennessee, have both been working for a number of years on a model of mammary neoplasia in the Sprague-Dawley rat. This model has been particularly valuable for evaluating the effects of LET, dose and dose rate on carcinogenesis. The system has been particularly useful for estimating RBE values at low doses, an essential function of a model for evaluating the dual radiation-action theory for radiation damage [10, 11].

Shellabarger has also used beams of neon ions of the BEVALAC to irradiate Sprague-Dawley rats. He exposed a total of 150 rats to neon doses of 2, 6, 18 and 54 rads. There were also 76 unirradiated controls. Ten months after irradiation, mammary tumours were found, primarily fibroadenomas, in each of these dose groups. In Fig. 2 we show Shellabarger's data for the 10-month period after exposure. The straight line through the five data points is a visual fit.¹ Although the percentage of rats with mammary tumours in the lower two dose groups (2 and 6 rads) is probably not significantly higher than in the unirradiated controls, there is no question that there is a marked increase in tumours following 18 and 54 rads. Following the latter dose group, more than 50% of the animals had a mammary tumour. In Vogel's rats, no tumours were palpable after doses up to 50 rads.

It is difficult to explain the difference in results between the Shellabarger and Vogel data. Both were carried out at the same facility, with the same ions, and the 'same strain' of rats. There may, however, be differences due to (a) biological or (b) physical factors in the two experiments. Although both investigators used 'Sprague-Dawley rats', the animals were obtained from different sources. Shellabarger purchased rats from Taconic Farms in the eastern United States of America, Vogel from Simonsen in California. It is well known that rat strains differ in genetic constitution and even a 'standard' Sprague-Dawley strain is not inbred and may develop sublines that show marked differences. Vogel has recently evaluated the influence of genetics and animal age on mammary tumour incidence in different rat strains [12]. There is certainly a genetic factor involved. Perhaps it may partly explain the different results of the two experiments. There are also differences in physical factors involved: Shellabarger exposed his rats in an anterior-posterior axis, facing and parallel to the beam of neon. In Vogel's set-up, the neon ions passed through the rat's body from right to left, the animal being perpendicular to the beam. The site of irradiation in the bio-medical curve facility also differed in Vogel's first experiment (2–50 rads), although in Experiment B, both investigators used the same irradiation site; and tumours were found in both groups of rats. It will be necessary to duplicate this low-dose work in the future to determine which factors are important.

1.4. Conclusions

- (a) Neon ions induce mammary tumours in Sprague-Dawley rats.
- (b) At low doses, neon ions are not nearly as efficient as fission neutrons in the induction of mammary neoplasia.
- (c) To produce a mammary neoplasm in 50% of irradiated Sprague-Dawley rats requires approximately 50 rads of neon and 150 rads of 250 kVp X-rays (CJS data): RBE – about 3.0.

¹ We express our appreciation to C.J. Shellabarger for these unpublished results.

- (d) By 300 days after exposure, 100 rads of neon do not induce significantly more mammary neoplasms than 100 rads of X-rays (HHV data).
- (e) The LET for fission neutrons at the DOSAR Reactor is $50 \text{ keV}/\mu\text{m}$, and the LET of neon ions at the BEVALAC facility is about $30 \text{ keV}/\mu\text{m}$. This difference in LET apparently is involved in the induction of mammary neoplasms in this female rat.

2. NEUTRON IRRADIATION: SINGLE VERSUS PAIRED DOSES

2.1. Source of irradiation

The DOSAR Health Physics Reactor at Oak Ridge National Laboratory, an unshielded fast reactor of the Godiva type, was used as the neutron source for irradiating rats. The reactor core is cylindrical, 22.86 cm high and 20.32 cm in diameter. Neutron energies were those of a slightly modified ^{235}U fission spectrum with an average energy of 1.2 MeV [9].

The neutron-to-gamma dose ratio was approximately 7. The neutron fluence, and indirectly the tissue kerma in free space, was monitored during each exposure by sulphur-pellet activity. The factors relating induced radioactivity to kerma were derived from measurements made with Hurst Threshold Detectors.

For each whole-body irradiation, 10 rats were placed in an exposure cage which was positioned at the centre of a rotating table [4]. With the reactor exactly 2 m above the animals, the reactor was 3.55 m above the floor in this position. The reactor was usually run at 1 kW of power, at a neutron dose rate of 8 rads/min.

2.2. The theoretical model

In 1976 J.W. Baum of Brookhaven National Laboratory proposed a mathematical model which postulated that cancer induction is a multi-event process; that these events occur naturally, usually one at a time in any cell; and that radiation frequently causes two of these events to occur simultaneously [13]. Kellerer and Rossi have shown, from microdosimetric considerations, that for high LET radiations the simultaneous events are associated with a single particle or track [14].

Baum's model predicts: (a) Linear dose-effect relations for early times after irradiation with small doses; (b) approximate power functions of dose (D^x) having an exponent less than one for populations of mixed age examined at short times after irradiation with small doses; (c) saturation of effect at either long times after irradiation with small doses or for all times after irradiation with large doses; and (d) a net increase in incidence which is dependent on age of observation,

TABLE I. SINGLE VERSUS SPLIT NEUTRON DOSES IN THE INDUCTION OF MAMMARY NEOPLASMS IN SPRAGUE-DAWLEY FEMALE RATS

Date of irradiation	Neutron doses (rads)	Number of rats exposed	Experiment terminated months after irradiation
31 August 1976	20	30	12
	10 ——— 10 ^a	30	
	0	20 (mobile, unirradiated controls)	
27 January 1977	70	40	11
	35 ——— 35 ^a	40	
	0	20 (mobile, unirradiated controls)	
1 April 1977	10	40	12
	5 ——— 5 ^a	40	
	0	22 (mobile, unirradiated controls)	

^a In the three split-dose experiments, one day (24 h) was the interval between the two neutron exposures.

but independent of age at irradiation. Vogel and Zaldívar's data for neutron-induced mammary tumours in rats [7] were used by Baum to illustrate the validity of the proposed model.

In his discussion of this theory, Baum suggested a test to compare single- and split-dose experiments. The model predicted equal incidences for these experiments if doses were below about 20 rads (total), but equal or greater effects from the split doses than from single doses for doses above about 50 rads. Previous data on neutron-irradiated mice agree with this prediction, whereas data on gamma-irradiated mice do not [15]. Gamma effects on rats [16, 17] draw somewhat greater effects for acute than fractionated doses at 400 days post-irradiation, but less significant differences at other times.

2.3. Experimental test

To test the single- and split-dose comparison mentioned by Baum, we have carried out the three neutron experiments illustrated in Table I. In these experiments, 110 young, adult, female Sprague-Dawley rats were exposed to single

TABLE II. INDUCTION OF MAMMARY TUMOURS 11¹ OR 12² MONTHS AFTER IRRADIATION

	20 rads ²	10—10 rads	No. of mammary tumours in unirradiated controls
Number of rats with mammary tumours/number alive one year after exposure	10/29 34%	15/29 ^a 52%	0/20
	70 rads ¹	35—35 rads	
	20/37 54%	22/35 63%	2/20
	10 rads ²	5—5 rads	
	10/38 26%	6/40 15%	0/22
			Total 2/62 = 3%

^a Includes one mammary tumour palpated then regressed.

neutron, whole-body irradiation; 110 rats were exposed to equal split doses with fractions given at the DOSAR Reactor, Oak Ridge National Laboratory, 24 h between doses; and 62 rats were unirradiated, mobile, control animals for the three experiments (see Table II).

A χ -square goodness of fit test was carried out to compare the induction of mammary tumours (%) in these three neutron experiments comparing single versus split doses of neutrons:

$$\begin{array}{r} \chi^2 \\ \text{Experiment 1} - 1.75 \\ \quad \quad \quad 2 - 0.57 \\ \quad \quad \quad 3 - 0.915 \end{array}$$

Since a χ^2 figure of 3.84 is necessary to show a significant difference (at the 5% level), there was no significant difference between the single- and paired-neutron doses in the induction of mammary tumours in the young, adult, female Sprague-Dawley rat under these experimental conditions. Thus, these three experiments do not prove or disprove the Baum hypothetical model [13].

2.4. Discussion

Shellabarger has recently carried out an experiment using neutrons of 0.43 MeV energy. He compared single and divided doses for the induction of mammary neoplasia in female, Sprague-Dawley rats. In these experiments, one neutron dose, single and divided, was utilized; but several time intervals (from minutes to months) were placed between the two paired fractions. In no case was a significant difference found between the single and paired doses of neutrons in inducing mammary neoplasia in these rats. These data were to be presented by Shellabarger at the Radiation Research Society meeting held in Toronto, Canada, May 1978 (personal communication).

In 1966 the Brookhaven group of investigators reported results on fractionation and protraction of total-body radiation on rat mammary neoplasia [16]. They found that female, Sprague-Dawley rats showed a high incidence of mammary neoplasia and a large number of mammary neoplasms following exposure to a single dose of 500 R of ^{60}Co gamma total-body irradiation. When studied over the entire lifespan, these responses were not different when the 500 R were given at either 40 or 160 days of age. Fractionation and protraction of this dose into 4×125 R (over 2 weeks) or 8×62.5 R (4 weeks) or 16×31.25 R (8 weeks) or 32×15.625 R (16 weeks) did not change the incidence of mammary neoplasia in rats or the total number of mammary neoplasms compared with a single dose of 500 R [16]. However, an increased yield of adenocarcinomas was reported.

Lamson and co-workers have also summarized the effect of single and divided doses of X-ray irradiation on rats [18]. They observed longevity in Long Evans-Wistar F_1 hybrid female rats after 0, 120, 240 and 480 R of total-body X-rays. These were administered either as single or divided exposures. The divided exposures consisted of either three or six equal fractions spaced at 3.5-, 7- or 14-day intervals. An increase in the number of dose fractions from 3 to 6 significantly improved longevity. The protective effect of dose fractionation also appeared to be dose dependent. Spacing of dose fractions at 3.5-, 7- or 14-day intervals did not influence longevity of the rats.

It would appear possible from Baum's theory that chronic irradiation at a low dose rate might produce a significantly greater effect (in inducing mammary tumours) than acute irradiation in the dose region above 20 rads. This could happen since

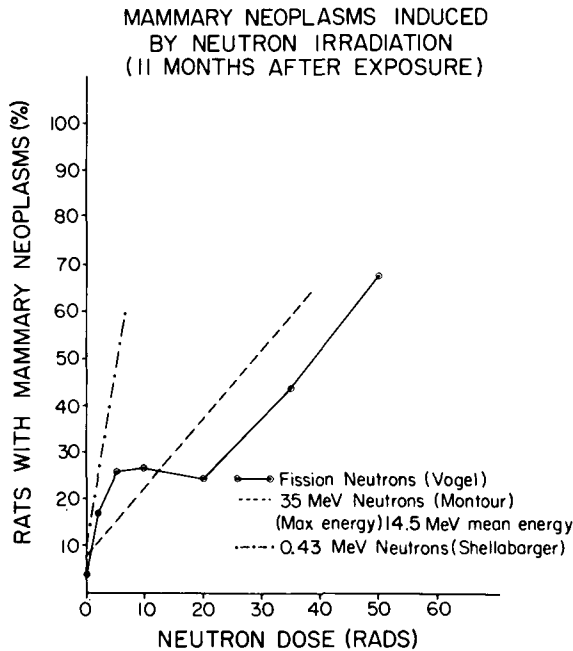


FIG.3. Mammary neoplasms induced by neutron irradiation of Sprague-Dawley rats. Comparative data of Vogel, Montour, and Shellabarger eleven months after irradiation (neutron mean energy: 1.2, 14.5 and 0.43 MeV).

damaged cells may be more apt to survive and permanently incorporate carcinogenic events while repairing other damage if additional injury is not imparted too soon. We plan to carry out a chronic neutron exposure of rats, at low neutron doses, over a period of a month or two, to determine the comparative effects of chronic versus acute neutron irradiation on the incidence of mammary tumours in the rat.

3. A COMPARISON OF NEUTRON/X-RAY RBE VALUES FOR THE INDUCTION OF MAMMARY NEOPLASMS IN SPRAGUE-DAWLEY RATS

3.1. Comparison

There have been at least four different experiments recently reported in which investigators have used neutrons of various energies to study the induction of mammary tumours in the Sprague-Dawley rat. We have compared the results of three of these experiments in Fig. 3. In this graph we have indicated the

TABLE III. NEUTRONS/X-RAYS RBE FOR MAMMARY TUMOUR INDUCTION IN SPRAGUE-DAWLEY RATS

Investigator	Neutron energy	RBE	
Montour	35 MeV (max.) neutrons mean = 14.5 MeV	at 40 rads = 5.0	at 2.5 rads = 13.8
Shellabarger	0.43 MeV neutrons	at 6.4 rads = 26.5	at 0.1 rad = 103
Vogel	Spectrum of fission neutrons from DOSAR Reactor (mean = 1.2 MeV)	at high doses at 5 rads 2 rads	~4 between 20 and 60 ~50
Broerse and co-workers	15 MeV/X-rays	20% mammary tumours 26.5 rads and 6.4 rads	RBE = 4.1
	0.5 MeV	20% level tumour prevalence	RBE = 12

TABLE IV. HIROSHIMA-NAGASAKI LEUKAEMIA CALCULATIONS (Barendsen)

 $A_1\gamma/A_2\gamma = 50$ rads: $A_1\gamma = 0.81$ $A_1n = 59$
leukaemia deaths per 10^6 person-year-rad $RBE_n = 73$ at low doses $A_1\gamma/A_2\gamma = 100$ rads: values of $A_1\gamma = 1.20$ and $A_1n = 57$ leukaemia deaths per 10^6 person-year-rad RBE_n value of 48

percentage of rats with mammary tumours 11 months after neutron exposure. We have chosen this time somewhat arbitrarily, since the percentage of mammary tumours in unirradiated, control rats of this strain in all three experiments is only 4–10% by eleven months.

The Brookhaven experiments (Shellabarger and co-workers) used 0.43 MeV neutrons at very low doses (maximum 6.4 rads) [6]. Vogel exposed his rats at the Health Physics DOSAR Reactor at the Oak Ridge National Laboratory and at Argonne National Laboratory's CP-5 Research Reactor [3]. The neutron spectrum at ORNL was a slightly modified fission spectrum from an unshielded reactor. The spectrum had a mean at 1.2 MeV. The LET of the DOSAR spectrum has been estimated as 50 keV/ μ m [9]. Montour used the Naval Research Laboratory's isochronous cyclotron in Washington, D.C. A neutron spectrum with a mean energy of 14.5 MeV was produced by the interaction of a 35-MeV deuteron beam on a thick beryllium target [19, 20]. The maximum energy of the neutrons was 35 MeV. (Montour's graphs [19] are labelled 35 MeV, but would be more accurately defined by the mean neutron dose of 14.5 MeV.)

Shellabarger's [16] and Montour's [19] data apparently indicate a linear relationship between dose and effect similar to that reported by Bond and co-workers for low LET radiation [21]. Vogel's data agree above 20 rads of neutrons, but show a non-linear relationship below this dose level.

In Table III we have compared the results of these three investigators with recently reported experiments by Broerse and co-workers [22–24] using 15 MeV and 0.5 MeV neutrons compared with X-rays for this same biological system in the Sprague-Dawley rat. The Relative Biological Effectiveness (RBE) of high versus low LET radiations (neutrons, X-, or gamma rays) is listed in the last column of this Table.

3.2. Conclusions

- (a) It is clear from all four of these experiments that the RBE increases as the dose is lowered. This value may reach 50–100 at very low neutron doses (Shellabarger and Vogel) [25].
- (b) In the three experiments using high neutron doses, the RBE was found to be between 4 and 5.
- (c) Montour reports an RBE (n/γ) of 13.8 (at 2.5 rads). Broerse reports an RBE (n/χ) of 12.0 at '20% level' tumour prevalence.
- (d) On the basis of the slope of the linear dose-response relationship, the 0.43 MeV neutrons were approximately 4.7 times as effective as were the 35 MeV (maximum) neutrons in producing mammary neoplasms [19].

3.3. Discussion

This biological system (of mammary tumour induction in the Sprague-Dawley rat) has stimulated much radiobiological controversy. Rossi and Kellerer [11], analysing some of Vogel's data, calculated that the induction of mammary neoplasms in this strain of rat was dependent on the action of neutrons on more than one cell. Thus a clonal theory of radiation carcinogenesis evolved. Recently, Gould and co-workers at Wisconsin have published results that conflict with these conclusions [26]. They claim that 'there appears to be no reason to reject the simplest hypothesis that one transformed cell is sufficient to produce a mammary tumor'. Montour and co-workers [19] support these conclusions, although they admit that 'the data analyzed in this paper are insufficient to prove conclusively that radiation-induced breast tumors in the Sprague-Dawley rat arise from either one cell or more than one cell'. Yet, these authors find 'no reason at this time to reject the single-cell hypothesis in favor of a multi-cell hypothesis, since a linear relationship adequately describes the data' [19].

Barendsen [27], in this symposium, has reported on the fundamental aspects of cancer induction in relation to the effectiveness of small doses of radiation. In calculating the relative biological effectiveness of neutrons versus gamma rays in the induction of human leukaemia, this investigator reports an RBE_n value of 48 at relatively high doses and 73 at low doses [28] (see Table IV). He also calculates an RBE_n value of 18.5 derived by assuming a simple linear relationship without any influence of cell reproductive death [28]. Barendsen's calculations are based on human leukaemia deaths per 10^6 person-year-rad. Comparisons are made between the Hiroshima and Nagasaki data (in which the neutron component differed significantly). This high RBE at low neutron doses supports the data reported in this paper for rat mammary tumour induction. We believe that the 'quality factor' of 10 for neutron irradiation effects in man needs further study.

There is evidence that, particularly at low neutron doses, the values should be elevated. (It is particularly at low neutron doses that most human exposures will occur.) We urge both national and international committees to study the neutron RBE values in different biological systems.

The causative agents in rat mammary carcinogenesis are not fully understood today. Some investigators still suspect that a virus is involved in the aetiology of this tumour. This has not yet been proved in the rat. Yokoro and co-workers [29] have discussed the role of prolactin in rat mammary carcinogenesis. These investigators have detected carcinogenicity of low-dose carcinogens and of persisting dormant cancer cells. The results of Shellabarger and Broerse also show that female sex hormones may be significant in this neoplasm.

The problem of the risks and benefits of human mammography is a controversial issue today. Bailar has written initially on the pros and cons of screening for early breast cancer [30]. He has discussed the radiation hazards of X-ray mammography at this conference [31]. We believe strongly that the radiobiological work on the irradiation of the rat mammary gland may have significance in assessing radiation risks in man.

ACKNOWLEDGEMENTS

We appreciate the excellent technical help during these experiments of our histological research technicians, Wilma Thaxton Martino and J. Russell Berry. We also wish to thank the operators of the DOSAR Reactor at Oak Ridge National Laboratory, Don Ward (now retired) and Mr. Gilley; and especially to Jerry Howard and Robert Springsteen for help at the BEVALAC facility.

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DISCUSSION

A.L. CARSTEN: The difference between your results and those of Shellabarger using neon ions is interesting. If the effect is a physics or dosimetry problem, are you going to repeat the study using Shellabarger's exposure geometry? Also, how does your low-LET information compare with Shellabarger's results?

H.H. VOGEL: I plan to repeat the low-dose neon experiment (2–50 rads) using Sprague-Dawley rats both from Simonsen (California) (HHV) and from Taconic Farms (CS) and using Shellabarger's exposure position for comparative values. Thus we may be able to determine whether biological or physical factors (or both) account for our different data.

Shellabarger's and Vogel's low-LET values are almost identical (see Ref.[5] of the paper, where the data are graphically compared).

J.J. BROERSE: With reference to the difference in tumour incidence after neon irradiation observed by you and Shellabarger, it seems useful to recall that our Sprague-Dawley (SD) strain has shown a longer latency period for spontaneous

tumour induction than noted by the American groups. In the near future we intend to compare the mammary tumour incidence after DMBA administration in the SD animals used at Brookhaven and at TNO. It could be useful to include your animals in the same experiment.

As you have referred to our results, I feel I should mention that our 1977 results were in part derived from macroscopic inspection of the animals during the observation period. The histological examination of the material of intact and sterilized animals has been completed, and this has resulted in slight changes in the original results. In fact, for the 0.5 MeV neutrons we now observe an RBE of 20 at the 30% prevalence level. The updated results confirm our initial conclusion of increasing RBE with decreasing dose.

Now I should like to ask two questions on your paper. First, the quality of neutron beams is described by rather complicated LET spectra. The concept of average LET has to be handled with caution. Could you tell us whether the quoted value of 50 keV/ μ m pertains to dose average or track average LET? Second, could you give some information about the relative proportion of histologically benign versus malignant tumours in the irradiated and control groups?

H.H. VOGEL: I think I can best answer your first question by quoting you the following reference: WILLHOIT, D.G., JONES, T.H., Dose and LET distribution in small animal-sized cylinders for a fission neutron spectrum, *Radiat. Res.* 44 2 (1970) 263. The work described in this paper was carried out at the DOSAR Health Physics Research Reactor, Oak Ridge, Tennessee, United States of America. This is the facility at which I exposed the rats to neutron doses. I believe this paper will answer some of your questions in regard to neutron dose and LET of this particular beam.

With regard to your second question, we have found that approximately 60–70% of our neutron-induced neoplasms in the Sprague-Dawley rats are adenofibromas, and about 30% adenocarcinomas (found early in the irradiated rats and late in unirradiated controls). Only a few fibrosarcomas were found. (See Ref. [7] of the paper.)

LONGEVITY STUDIES IN RHESUS MONKEYS AFTER X-RAY AND NEUTRON IRRADIATION with special emphasis on tumour induction

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Abstract

LONGEVITY STUDIES IN RHESUS MONKEYS AFTER X-RAY AND NEUTRON IRRADIATION WITH SPECIAL EMPHASIS ON TUMOUR INDUCTION.

Over the past 15 years experiments have been performed on the mortality of rhesus monkeys and the effects of bone marrow transplantation after irradiation with X-rays and fission neutrons. It had been previously established that, for neutrons as well as for X-rays, bone marrow transplantation was effective in protecting the animals from death due to the haemopoietic syndrome. Of the group of X-irradiated monkeys, 14 survived more than three years after irradiation, whereas in the neutron-irradiated group nine animals were long-term survivors. A control group of comparable age distribution comprises a total number of 13 non-treated rhesus monkeys. All animals are kept under continuous observation to study the late effects of radiation. After latent periods of 4 to 12 years, five neutron-irradiated and one X-irradiated animal died with tumours. The relation between the latency period, the radiation dose and the types and localization of tumours are discussed. The present results suggest relatively high effectiveness of fission neutrons for tumour induction.

INTRODUCTION

In order to assess the risks of exposure to ionizing radiation for man, it is useful to investigate the effects of irradiation in a mammalian species whose characteristic response to irradiation is similar to that of the human. For this reason, the non-human primate has been chosen as the experimental subject in which to study the effects of whole-body irradiation, as well as the effects of therapeutic measures, such as bone marrow transplantation.

An increasing number of patients are at present surviving following whole-body, high-dose irradiation and isogeneic and allogeneic bone marrow transplantation. Also, neutron irradiation is used with increasing frequency in radiotherapy. It is therefore important to collect information on the long-term risk for the development of malignancies from similar treatments in subhuman primates.

Since 1963, experiments have been performed on the mortality of rhesus monkeys and the effects of autologous bone marrow transplantation after irradiation with X-rays and fission neutrons. The experimental methods and the early radiation effects have been reported elsewhere [1], and will therefore only be summarized in the present communication. Of those monkeys which were irradiated and reconstituted with bone marrow, 14 animals survived more than three years following X-ray irradiation, whereas 9 survived longer than three years in the neutron-irradiated group. These long-term survivors were kept under continuous observation to study the late effects of radiation, and a relatively high mortality from malignant tumours was found in the neutron-irradiated group.

In this report we present the survival data, the latency period before the appearance of tumours, and the types and localization of the tumours occurring in the irradiated groups of monkeys.

EXPERIMENTAL METHODS

Rhesus monkeys (*Macaca mulatta*) were generally held for approximately one year in the stock colony (including three months quarantine) before they were used for the experiments. At the time of irradiation, they were about three years of age, and their weights varied between 2300 and 3000 g. The supraethally irradiated monkeys were grafted intravenously with 2 to 4×10^8 autologous bone marrow cells (in Hank's balanced salt solution) per kg body weight a few hours after irradiation.

Whole body X-irradiations were performed with a Philips-Müller generator (300 kV, HVL 3 mm Cu) with an average dose rate over the animal of 28 rads per minute. Neutrons with a mean energy of about 1 MeV were produced in a ^{235}U converter plate in the Low Flux Reactor (LFR) of the Netherlands Energy Research Foundation (ECN) at Petten. Detailed information on the design of the fast neutron facility and the exposure arrangements are published elsewhere [2]. The mean value for the gamma-dose contamination over the animals was equal to 24% of the total dose [1]. The irradiations were carried out at a mean dose rate of about 8 rads/min (total dose of neutrons and gamma-radiation). The doses received by the monkeys were expressed as the absorbed dose in soft tissue averaged over the animals.

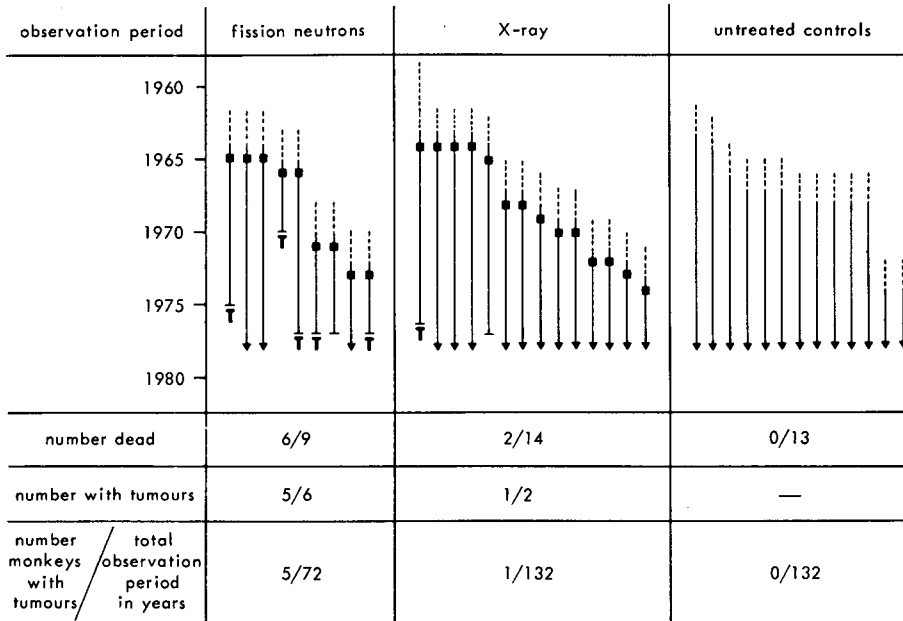


FIG.1. The tumour incidence and post-irradiation intervals are given for long-term surviving rhesus monkeys after whole-body irradiation and bone marrow transplantation. The total observation period for each group, including the untreated controls, is shown. Dashed portion of lines indicates approximate age of monkeys before entering colony; solid lines indicate years held under observation; ■ indicates time of irradiation; line ending in cross-bar indicates death; T indicates tumour; lines ending in arrowheads indicate monkeys still alive.

The acute effects of lethal irradiation in rhesus monkeys have been described [1], but pertinent to the present study is the following: Doses in excess of 525 rads X-rays and 260 rads fission neutrons produced 100% lethality in monkeys which did not receive bone marrow transplantation. For the lethally irradiated monkeys, a mean survival time of 12 days was observed. These animals died from the classical bone marrow syndrome. Autologous bone marrow transplantation increased the mean lethal dose from 525 rads to 925 rads for X-irradiated animals, and from 260 rads (total dose of neutron and gamma rays) to 470 rads for neutron-irradiated animals. For higher total doses, bone marrow grafting was no longer effective and the animals died with severe intestinal damage [1].

The two groups of long-term surviving animals consisted of nine monkeys irradiated with fission neutrons, in doses ranging from 230 to 440 rads, and fourteen X-irradiated monkeys which received doses ranging from 400 to 860 rads.

TABLE I. PATHOLOGY DATA OF LONG-TERM SURVIVING RHESUS MONKEYS AFTER NEUTRON AND X-IRRADIATION

Case No.	Sex	Dose and type of radiation	Autologous BM transplant	Post-irradiation interval (years)	Cause of death	Significant histological findings
1	M	380 rads fission neutrons	yes	4	Neoplasia	1. Malignant glomus tumour, s.c. of chin, metastatic to liver 2. Osteosarcoma, humerus
2	F	440 rads fission neutrons	yes	4	Neoplasia	1. Glioblastoma, temporal lobe
3	F	380 rads fission neutrons	yes	6	Unknown	(Non-neoplastic, degenerative lesions in various organs)
4	M	410 rads fission neutrons	yes	6	Neoplasia	1. Astrocytoma, fronto-parietal region 2. Ossifying fibroma, skin
5	M	230 rads fission neutrons	no	10	Neoplasia	1. Malignant glomus tumour, pelvic cavity, metastatic to lungs 2. Islet cell adenoma
6	M	350 rads fission neutrons	yes	11	Neoplasia, sepsis	1. Papillary cystadenocarcinoma, kidney 2. Osteosarcoma, skull 3. Splenoma
7	M	860 rads X-ray	yes	12	Neoplasia	1. Malignant glomus tumour, s.c. of elbow, metastatic to lungs, kidneys, meninges and skull 2. Osteosarcoma, maxilla
8	F	430 rads X-ray	yes	12	Haemoperitoneum	1. Endometriosis

In addition, a group consisting of thirteen untreated rhesus monkeys of a comparable age distribution was maintained under identical conditions of housing and nutrition to serve as a control group.

A complete necropsy was done on all cases as soon as possible following death of the animal. Tissues were fixed in neutral buffered formalin, embedded in paraffin, and sectioned at 5 μ m. Slides were stained with haematoxylin-phloxine-saffron (HPS) and special stains where indicated.

TUMOUR INCIDENCE AND LATENCY PERIOD IN SURVIVORS

The data on longevity and tumour incidence in the three groups of monkeys are given in Fig.1. Six of the neutron-irradiated monkeys have died to date, five as a result of neoplasia. These monkeys, four males and two females, died between 4 and 11 years following irradiation. Two X-irradiated monkeys, a male and a female, died 12 years after irradiation, one of these dying (at 18 years of age) as a result of neoplasia. At the time of writing, none of the control group has died.

Although the numbers in each group are relatively small, the tumour incidence in the neutron-irradiated group (average dose received by the total group, 340 rads) was 56%, and 7% in the X-irradiated group (average dose, 750 rads), disregarding the post-irradiation intervals.

The results can also be examined in terms of the number of animals developing tumours per group as a function of the total observation period for the entire group. Thus, in the neutron-irradiated group, five monkeys developed tumours in a total observation period of 72 monkey years (Fig.1). In the X-irradiated group, however, only one animal died with tumours in a total observation period of 132 monkey years.

The post-irradiation intervals for the neutron-irradiated monkeys which died varied between 4 and 11 years. There is some indication for an inverse correlation between the time until death for the five monkeys with neoplasia and the dose of neutron irradiation. Three of four animals receiving the higher doses (380 to 440 rads) of neutrons died between 4 and 6 years following irradiation; the fourth animal, which had received 380 rads neutrons, died 6 years after irradiation without histopathological evidence for neoplasia. The two monkeys dying with tumours 10 and 11 years after irradiation had received lower doses of neutrons, namely 230 and 350 rads.

The post-irradiation interval for the two X-irradiated monkeys which died was 12 years. In this instance, there was no apparent effect of a twofold difference in radiation dose on the time until death following irradiation.

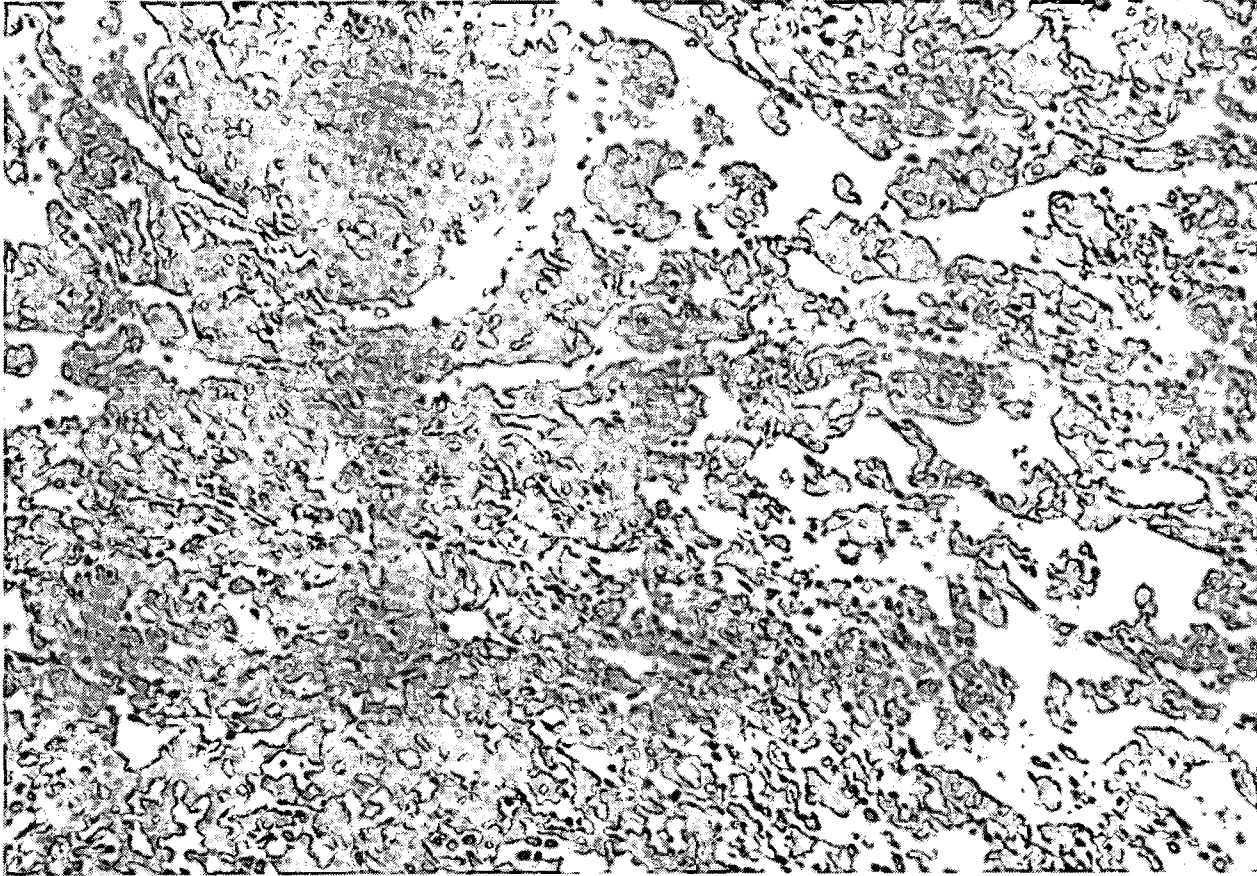


FIG.2. Malignant glomus tumour of male rhesus monkey (case 5) which died 10 years after irradiation with 230 rads fission neutrons. Note the numerous irregular vascular spaces lined by cords and sheets of uniform-appearing tumour cells. (HPS, $\times 210$)

Since only one of these monkeys died with neoplasia (the oldest from its group), no conclusions can be reached at this time regarding the relationship between dose of X-radiation and the latency period for tumour development.

PATHOLOGICAL FINDINGS

The most pertinent pathological changes in these monkeys, primarily those of a neoplastic nature, are briefly summarized in Table I.

Malignant glomus tumours were found in three monkeys, two of which had been irradiated with neutrons (cases 1 and 5), and one irradiated with X-rays (case 7). The primary tumour was located in the subcutis of the chin in one case, and of the elbow in the second. In the third case, a subcutaneous glomus tumour was removed from the sacral region. At necropsy approximately 10 months later, a large tumour mass was found in the pelvic cavity, adherent to and invading the acetabulum. No signs of local recurrence were observed in the sacral region, and no conclusion was reached as to which of these sites constituted the primary location of the tumour. Metastatic tumour foci were found in all three cases (see Table I for sites of metastases).

The histological features of these tumours in the monkey are identical with those described for glomus tumours of man [3-5]. All three tumours are characterized by the presence of numerous vascular channels of variable size surrounded by cords and sheets of uniform 'epithelioid' cells, with pale cytoplasm and prominent round-to-oval nuclei (Fig.2). In one case (case 7), electron microscopic examination was carried out and revealed features similar to those previously described [3-5]. Briefly, these include the presence in the tumour cells of abundant pinocytotic vesicles, cytoplasmic microfilaments, dense plaques beneath the cell membrane, and abundant intercellular basement membrane material.

Osteosarcomas were found in three monkeys, two of which were irradiated with neutrons (cases 1 and 6), and the third with X-rays (case 7). They were located in the proximal humerus, the anterior portion of the calvarium and in the maxilla. It is interesting that in two cases (cases 1 and 7) the osteosarcomas occurred concurrently with malignant glomus tumours. In one case, the osteosarcoma was classified as an osteoblastic type, characterized by proliferation of malignant osteoblasts which produced variable amounts of osteoid (Fig.3). In another case, the tumour contained numerous prominent vascular spaces, and was classified as a telangiectatic type. In the third case the tumour was characterized by sheets and fascicles of elongated spindle-form cells, with scanty osteoid deposition. This tumour is compatible with a fibroblastic type of osteosarcoma.

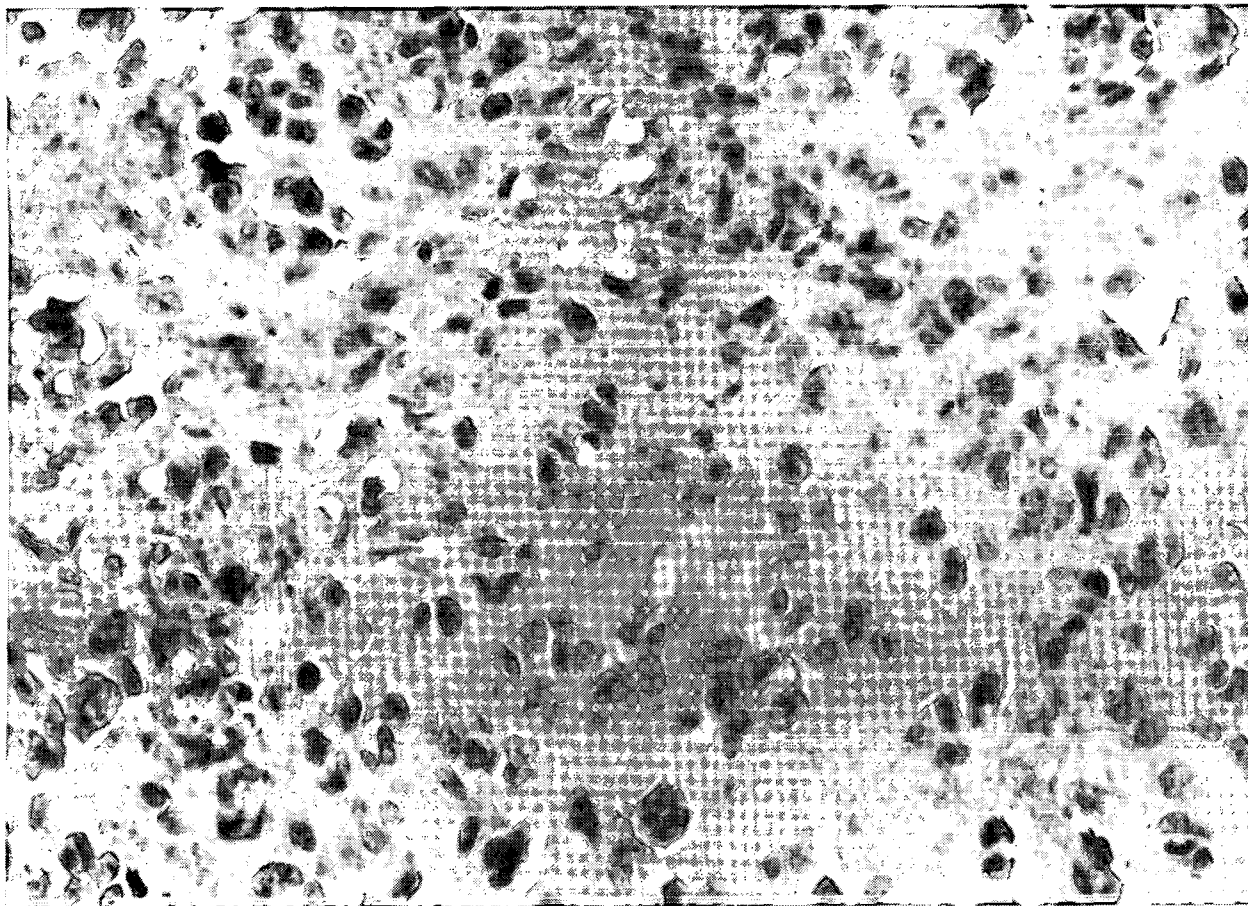


FIG.3. Osteosarcoma of maxilla of male rhesus monkey (case 7) irradiated 12 years previously with 860 rads X-rays. The malignant, pleomorphic osteoblasts are surrounded by abundant osteoid. (HPS, $\times 500$)

In two monkeys, both irradiated with neutrons, central nervous system tumours developed (cases 2 and 4). In one monkey, there was a tumour mass in the temporal lobe. The tumour was highly cellular and composed of pleomorphic glial cells, including bizarre multinucleated giant cells. Mitoses were common. Pseudo-palisading of tumour cells was seen around areas of necrosis (Fig.4). The histological appearance was compatible with glioblastoma multiforme. In the second case, the tumour was located in the fronto-parietal region, and was characterized grossly by an ill-defined white mass, with a gelatinous central cavitation. Histologically, the tumour was compatible with an astrocytoma (Fig.5).

One neutron-irradiated monkey (case 6) developed a tumour of the kidney. The kidney was approximately twice the normal size, and contained, in addition to the friable, necrotic tumour mass, numerous cysts of varying size. Histologically, the neoplasm was a papillary cystadenocarcinoma, with extensive necrosis, purulent inflammation and fibrosis. In addition to the kidney tumour, this animal also had an osteosarcoma of the calvarium (mentioned above), and a splenic nodule consistent with a splenoma.

Other neoplasms found in the neutron-irradiated monkeys were an ossifying fibroma of the skin (case 4), and a pancreatic islet cell adenoma (case 5).

One of the two X-irradiated monkeys (case 8) died as a result of massive haemoperitoneum secondary to severe external endometriosis. The mass of ectopic endometrial tissue incorporated the ovaries, Fallopian tubes, most of the uterus, and was adherent to the colon and mesentery. No evidence of neoplasia was found in this animal.

Finally, one neutron-irradiated monkey (case 3) died 6 years after irradiation without tumours. This animal had several relatively mild degenerative lesions in a number of organs, none of which were judged significant enough to cause the animal's death.

DISCUSSION

The group of 23 monkeys surviving more than three years following irradiation and bone marrow transplantation, form an interesting group of animals in which to study the long-term effects of irradiation.

Since so few monkeys in the X-irradiated group, and none in the control group, have died up to the present time, the data presented here must be considered preliminary, especially as regards information on tumour incidence. However, some comments appear to be justified regarding the relative biological effectiveness (RBE) for tumour induction by neutron versus X-ray irradiation, since obvious differences exist between the groups.

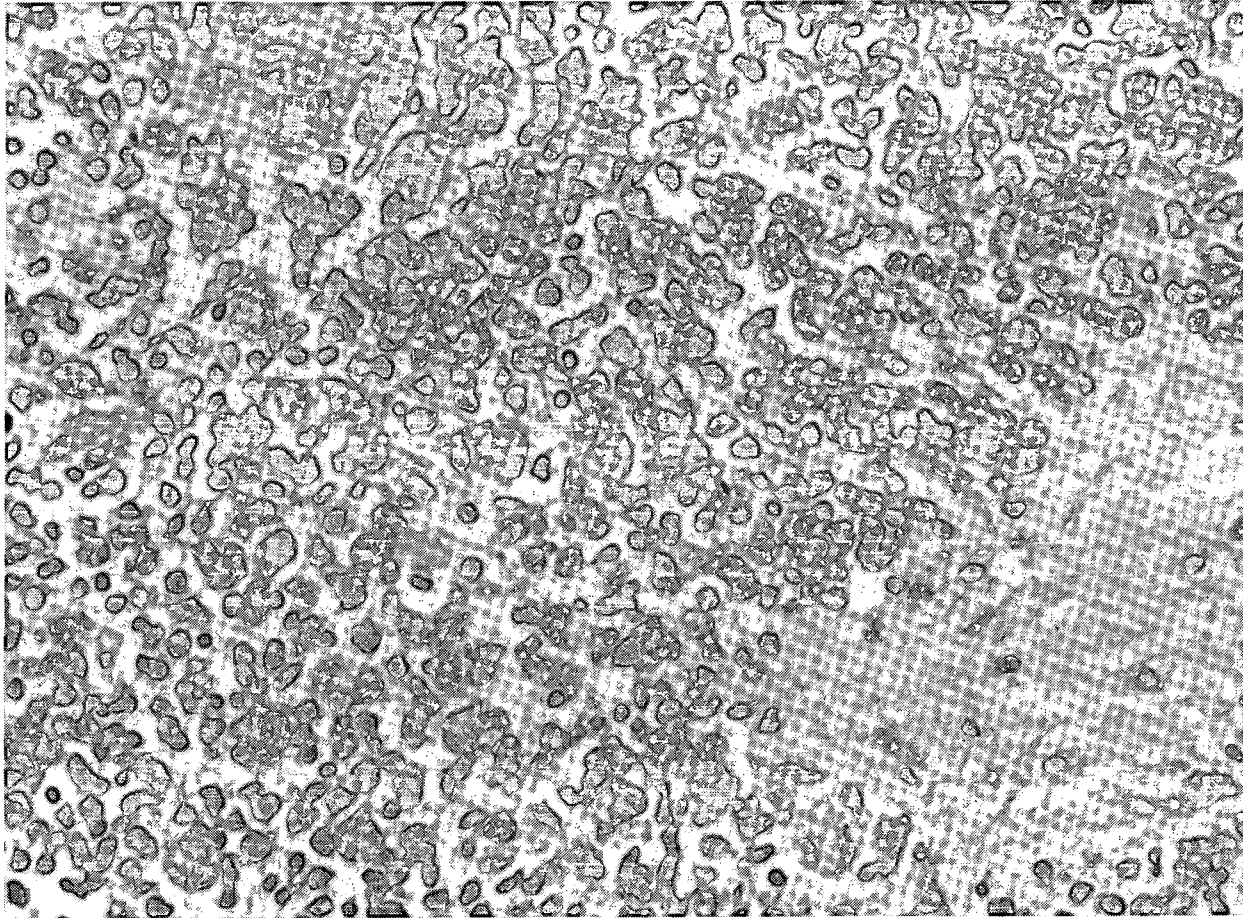


FIG.4. Glioblastoma multiforme in temporal lobe of female rhesus monkey (case 2) irradiated 4 years previously with 440 rads fission neutrons. The neoplastic glial cells partially surround a necrotic area (lower right); note the high degree of pleomorphism of the tumour cells. (HPS, $\times 500$)

In a total observation period of 72 monkey years, five of nine neutron-irradiated monkeys (average dose 340 rads) died with neoplasms and only one of 14 X-irradiated monkeys (average dose 750 rads) during a total observation period of 132 monkey years. In a comparable period, 132 monkey years, no control animal has died from neoplasia. It is of interest to compare our preliminary results with estimates made for induction of neoplasms in man: $5 \times 10^{-6} \text{ year}^{-1} \text{ rem}^{-1}$ for all types of cancer [6]. From our results we would derive a value of $1/132 \times 750 = 10 \times 10^{-6} \text{ year}^{-1} \text{ rad}^{-1}$ for X-rays and $5/72 \times 340 = 200 \times 10^{-6} \text{ year}^{-1} \text{ rad}^{-1}$ for fission neutrons. It should be realized that these values may be an underestimation, since a number of the irradiated monkeys developed multiple tumours. In any event, a comparison of these risk factors would indicate an RBE in excess of 10 for tumour induction after irradiation with fission neutrons.

The question of the influence of radiation on longevity is a complex one. Factors that are obviously important in determining length of survival following irradiation are inextricably linked to the type and dose of radiation, and include the types and behaviour of radiation-induced tumours, which must be considered against the background of spontaneous, age-associated neoplastic, as well as non-neoplastic diseases, about which very little is known in the non-human primate. In this communication, we have restricted ourselves to a brief account of the neoplastic diseases encountered in the irradiated monkeys. A description of the non-neoplastic degenerative and inflammatory lesions, which are obviously also of importance in a study of the biological effects of radiation, will be dealt with in a separate publication.

It is difficult to establish the precise relationship between ionizing radiation and the various neoplasms observed in these monkeys. Although, in general, reports of neoplasms in rhesus monkeys are relatively few in number, most of the neoplasms described by us have been previously reported, with a few exceptions. To our knowledge, the occurrence of malignant glomus tumours in non-human primates has not been previously described. Similarly, no reports on spontaneous central nervous system tumours in rhesus monkeys have appeared. A glioblastoma multiforme was reported in a *M. mulatta* which had received local gamma and thermal neutron irradiation to the head [7]. It is interesting that both central nervous system tumours in our series occurred in animals irradiated with the highest doses of neutrons (410 and 440 rads), and both died after relatively short latent periods (4 and 6 years). It appears that, in the rhesus monkey at least, primary central nervous system tumours may be more readily induced by neutron irradiation than by conventional X-rays.

Osteosarcomas and renal adenocarcinomas have been reported previously in both irradiated [8] and in non-irradiated rhesus monkeys [9,10].

The pathological data which we shall obtain from our group of control monkeys will be extremely valuable in the interpretation of the lesions found

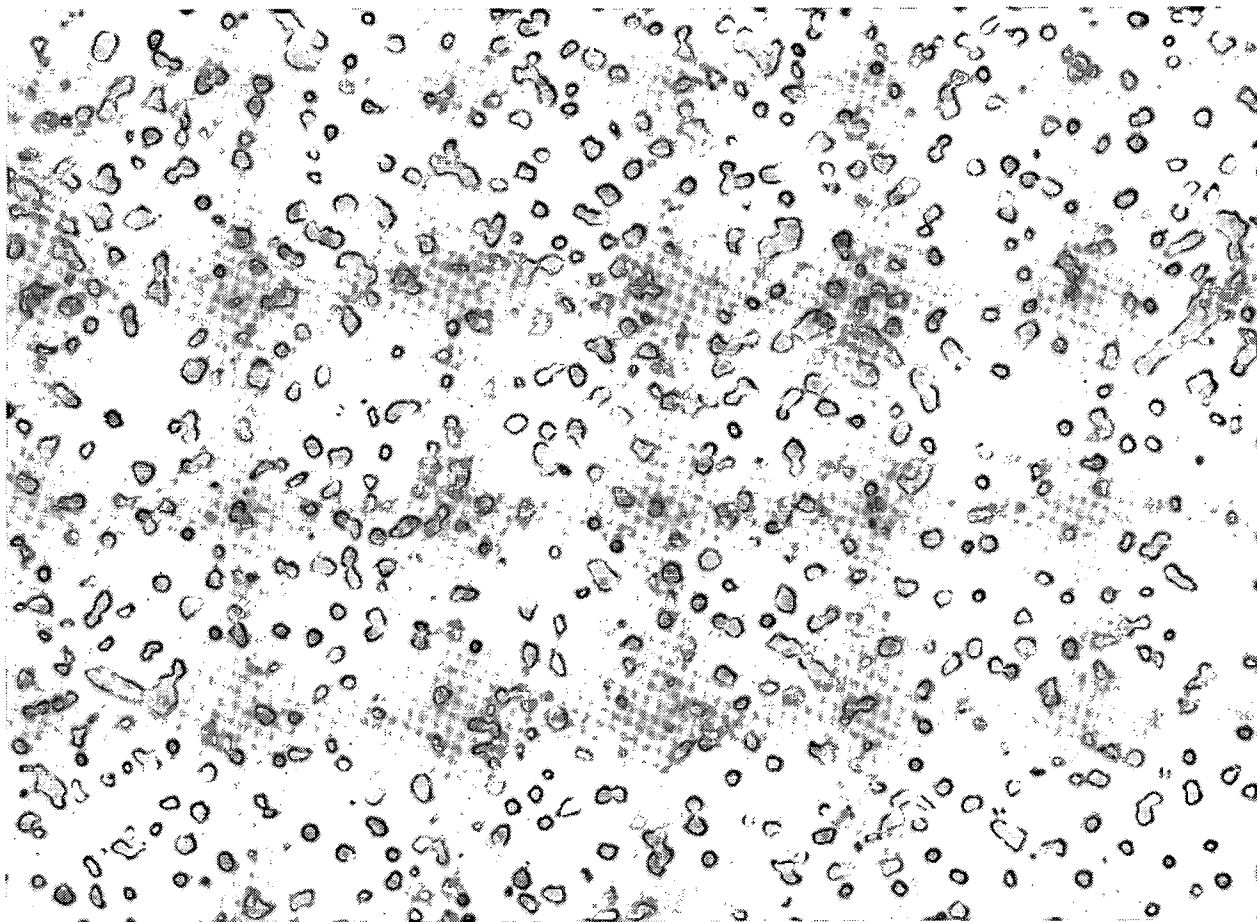


FIG.5. Astrocytoma from the fronto-parietal region of a male rhesus monkey (case 4) which died 6 years after irradiation with 410 rads fission neutrons. There is a uniform distribution of tumour cells, and prominent capillary blood vessels are present. (HPS, $\times 270$)

in the irradiated monkeys. The only information we have regarding the incidences of neoplasia in normal, untreated rhesus monkeys from our colony which died spontaneously concerns three females and a male, with a mean age of 16 years (range 14 to 20 years), which were derived from other studies. There was no evidence of neoplasia in these four monkeys.

Further information on tumour induction and longevity will be collected in the coming years, in particular for the groups of control and X-irradiated monkeys. However, the present results already indicate a relatively short latency period, and a relatively high effectiveness of fission neutrons for tumour induction compared with the results after X-irradiation.

ACKNOWLEDGEMENTS

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DISCUSSION

W. GÖSSNER: The glomus tumour which you have observed is a special type of vascular tumour, also called glomangioma or angiomatous glomus tumour, and is normally regarded as a benign tumour in human pathology. A corresponding malignant vascular tumour is the haemangiopericytoma. In this context I should like to ask the following questions: first, did all the glomus tumours you found exhibit the criteria of malignancy; second, how do you differentiate these malignant glomangioma from haemangiopericytoma; and third, did you also find this type of tumour in untreated rhesus monkeys?

M.J. van ZWIETEN: The answer to your first question is yes. Distant metastases were found in each of the three monkeys with glomus tumours. The tumours were diagnosed as glomus tumours on the basis of their morphologic similarity to those reported in man. In addition, in the case studied by electron microscopy, features were found which again conform to descriptions of glomus tumours in man, and these tend to exclude haemangiopericytoma as a diagnosis. In particular, the presence of large numbers of intracytoplasmic microfilaments and abundant basement membrane material around tumour cells, in addition to the other features of smooth muscle cells, reportedly tend to support more a diagnosis of glomus tumour.

As far as your third question is concerned, no, we have not found this type of lesion in our untreated monkeys.

H.H. VOGEL: I should like to comment on the high RBE (20 or above) that you have suggested for n/x values for induced neoplasms. This is one more bit of evidence for a biological system with high neutron RBE value (and not necessarily only at low doses such as the values I mentioned in my paper).¹

Now I have two questions, first, did you observe any radiation cataracts in the neutron-irradiated monkeys, and, second, in the acute radiation studies, is there any evidence in the monkey of the 'gastro-intestinal radiation syndrome' so characteristic of rats and mice irradiated with mid-lethal neutron doses leading to death usually between days 4 and 10? What is the pathology of the monkey G-I tract exposed to acute neutron doses?

¹ VOGEL, H.H., "High LET irradiation of Sprague-Dawley female rats and mammary neoplasm induction", these Proceedings **2**, IAEA-SM-224/233.

M.J. van ZWIETEN: We have observed cataracts in two of the six neutron-irradiated monkeys which have died to date. In addition, one of the three remaining neutron-irradiated survivors also has a cataract.

J.J. BROERSE: In our studies on the acute radiation effects in the monkeys, we observed that after fast neutron irradiation with total doses of 500 rads and higher, the bone marrow grafting was no longer effective and the animals died within seven days with severe damage to the small and large intestine. For irradiations with X-rays at doses of 1000 rads and higher a delayed intestinal syndrome was observed. This finding of earlier death after neutron irradiation could indicate that the degree of radiation damage in the intestinal tract of the monkeys is more severe after fast neutron irradiation than after X-irradiation.

NEOPLASMS IN DOGS RECEIVING LOW-LEVEL GAMMA RADIATION DURING PRE- AND POSTNATAL DEVELOPMENT

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Abstract

NEOPLASMS IN DOGS RECEIVING LOW-LEVEL GAMMA RADIATION DURING PRE- AND POSTNATAL DEVELOPMENT.

As a part of a long-term study to evaluate the biological hazards associated with low levels of ionizing radiation, beagle dogs were given whole-body exposure to ^{60}Co gamma radiation at one of six ages of pre- or postnatal life. Four-hundred and eighty dogs, 120 for each age at exposure, received 20 R at 8, 28 or 55 days *postcoitus* (*dpc*) or at 2 days *postpartum* (*dpp*). Similarly 480 dogs, in groups of 120, were exposed to 100 R at these same ages. Exposures of 100 R were also given to 118 dogs at 70 *dpp* and 231 dogs at 365 *dpp*. An additional 359 dogs were sham-irradiated. Both sexes were equally represented. The absorbed dose was calculated for each dog. Mean values for each age at exposure ranged from 15.6 to 17.5 rads for 20 R exposures and from 80.8 to 88.3 rads for exposure to 100 R. Mortality due to neoplasia during the initial ten years of the experiment was examined. Twenty dogs died or were killed because of neoplasia, 19 having been irradiated. Tumours in these 19 irradiated dogs included five malignant lymphomas, eight carcinomas (two of mammary origin, two of prostatic origin, and one each of oral mucosa, ovary, urinary bladder, and thyroid origin), four sarcomas (two haemangiosarcomas, one fibrosarcoma and one mast cell sarcoma), one astrocytoma, and one hepatocellular adenoma. Neoplasms occurred in all irradiated groups except 8 *dpc* (20 and 100 R) and 70 *dpp* (100 R). Eleven neoplasms developed in dogs irradiated perinatally (55 *dpc* or 2 *dpp*) with 20 or 100 R. Four of the tumours in the perinatally irradiated dogs were detected before two years of age. The earliest death was at three months, due to an astrocytoma. A single sham-irradiated dog died of a malignant tumour, a mammary carcinoma. Preliminary analyses point to three findings of particular interest: (1) the preponderance of neoplasms causing death or euthanasia occurred in irradiated dogs; (2) the unusual finding of four deaths due to neoplasia before two years of age in perinatally irradiated dogs; and (3) the occurrence of five malignant lymphomas in this relatively small irradiated population.

INTRODUCTION

The role of ionizing radiation in the induction of neoplasia is well established. Numerous studies in both man and animals have shown that exposure to high levels of radiation can lead to development of neoplasms in a variety of organ systems [1]. The role of relatively low levels of ionizing radiation in carcinogenesis is not so well established and there is uncertainty as to the importance of low-level exposure in man. Stewart and Kneale [2] first noted that low-level medical diagnostic radiation might be associated with cancer in children exposed *in utero*. Such epidemiological findings of increased cancer risk for children of mothers who received X-ray examination during gestation have been confirmed by some groups [3,4] but questioned by others [5,6]. Controversy still exists as to the significance of medical diagnostic exposures in relation to carcinogenesis in man.

While there is a considerable body of literature relative to the carcinogenic effects of high levels of ionizing radiation in experimental animals, there is relatively little information on low-level effects. Even less is available dealing with the effects of age on radiation carcinogenesis, especially with respect to prenatal exposures. This lack of information pertaining to a relatively long-lived animal model has made interpretation of the results of human epidemiologic studies uncertain.

The Collaborative Radiological Health Laboratory (CRHL) of Colorado State University and the Bureau of Radiological Health of the Food and Drug Administration have been conducting a long-term animal experiment for the purpose of determining the lifetime hazards associated with prenatal and early postnatal exposure to ionizing radiation. This is a life span study of a moderately large and long-lived mammal, the beagle dog, exposed at one of several times during development to a relatively small, discrete dose of external ^{60}Co gamma radiation. The major concern of the experiment is the evaluation of the role of age as a factor influencing an animal's response to radiation injury. This paper is a status report of the ongoing long-term experiment with specific reference to the neoplasms that have led to the death of experimental animals.

MATERIALS AND METHODS

The beagles used in this experiment are part of a closed colony which has had no new animals entered since 1964. The dogs are housed in outdoor kennels and fed dry kibbled dog food and water ad lib unless there is a medical reason to change the diet in individual dogs. All dogs are observed daily and given complete annual physical examinations. In case of illness the dogs are given more frequent examinations as required.

One-thousand-six-hundred-and-sixty-eight dogs received either a single whole-body exposure to ^{60}Co gamma radiation or a sham-irradiation. All exposures were bilateral for a total of 10 minutes. Dogs were exposed or sham-exposed at 8, 28 or 55 days *postcoitus* (*dpc*) or 2, 70 or 365 days *postpartum* (*dpp*). The exposure was either 20 R or 100 R as measured as the midline in-air dose. Detailed procedures for exposures and dosimetry have been reported [7]. The mean absorbed

TABLE I. EXPERIMENTAL DESIGN OF STUDY TO EVALUATE THE LONG-TERM EFFECTS OF WHOLE-BODY ^{60}Co GAMMA RADIATION IN BEAGLES

Age at exposure	Exposure (R)					
	0		20		100	
Prenatal exposures	L ^c	S	L	S	L	S
8 <i>dpc</i> ^a	40	20	80	40	80	40
28 <i>dpc</i>	40	20	80	40	80	40
55 <i>dpc</i>	40	20	80	40	80	40
Postnatal exposures						
2 <i>dpp</i> ^b	40	20	80	40	80	40
70 <i>dpp</i>	40	20	--	--	80	40
365 <i>dpp</i>	40	20	--	--	160	80
Number of dogs per level of exposure	240	120	320	160	560	280
Total number of dogs	1680					

a Days *postcoitus*.

b Days *postpartum*.

c L: Number of dogs scheduled for life span observation

S: Number of dogs scheduled for sacrifice at 5, 8, 11 or 14 years of age.

Equal numbers of males and females are present in both L and S groups.

doses for dogs exposed to 20 and 100 R at 8 *dpc* were 15.9 and 81.6 rads respectively; at 28 *dpc* they were 16.0 and 80.8 rads; at 55 *dpc* they were 15.6 and 80.8 rads; and at 2 *dpp* they were 17.5 and 88.3 rads. Dogs exposed to 100 R only at 70 *dpp* and 365 *dpp* had mean absorbed doses of 82.6 and 81.2 rads respectively. The overall mean dose for dogs exposed at 20 R was 16.3 rads with a range of 15.1 to 17.8 rads. The mean for dogs exposed at 100 R was 82.6 rads with a range of 75.3 to 93.8 rads. Equal numbers of males and females were used. The dogs were entered into the study over a 5-year period from 1967 through 1973. Two-thirds of the animals are being maintained for the duration of their natural lives while the remaining one-third are being sacrificed for sequential study at 5, 8, 11 and 14 years of age. Table I outlines the experimental design for the study.

TABLE II. CURRENT STATUS OF LONG-TERM EXPERIMENT TO EVALUATE THE EFFECTS OF WHOLE-BODY EXPOSURE OF BEAGLES TO ^{60}Co GAMMA RADIATION (AS OF JANUARY 31, 1978)

Age at exposure	Exposure (R)					
	0		20		100	
	L ^c	S	L	S	L	S
Prenatal exposures						
8 dpc ^a	34/40 ^d	12/20	73/80	27/40	75/80	24/40
28 dpc	31/40	11/20	70/80	22/40	70/80	28/40
55 dpc	28/40	13/20	76/80	24/40	66/80	24/40
Postnatal exposures						
2 dpp ^b	35/40	10/20	66/80	17/40	66/80	26/40
70 dpp	26/40	13/20	---	---	69/78	25/40
365 dpp	36/39	12/20	---	---	128/155	34/76
Totals	261/359		375/480		635/829	

a Days *postcoitus*.

b Days *postpartum*.

c L: Number of dogs scheduled for life span observation

S: Number of dogs scheduled for sacrifice at 5, 8, 11 or 14 years of age.

Equal numbers of males and females are present in both L and S groups

d Number of dogs alive/number of dogs alive at time of irradiation or sham-irradiation.

A number of endpoints are being evaluated in the beagles on this experiment including survival, growth and development, physiological functions and disease incidence patterns including neoplasia. Whenever feasible, neoplasms are removed surgically as soon as possible after clinical diagnosis. All dogs that either die, are euthanatized, or are sacrificed at predetermined ages are given complete gross and histopathological examinations. Tissues are fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm and stained routinely with haematoxylin and eosin. All diagnoses are made without prior knowledge of experimental groupings. Statistical evaluation of survival and disease incidence data are based on the method of Kaplan and Meier [8]. Data are expressed as cumulative probabilities of disease incidence.

TABLE III. SUMMARY OF DEATHS DUE TO NEOPLASIA IN BEAGLES EXPOSED TO WHOLE-BODY ^{60}Co GAMMA RADIATION (AS OF JANUARY 31, 1978)

Age at exposure	Exposure (R)	Number exposed	Number dead of neoplasia	Age at death (years)	Neoplasm
8 dpc ^a	20	120	0	---	
28 dpc	20	120	1	6.1	Mast cell sarcoma, skin
55 dpc	20	120	1	7.6	Malignant lymphoma
2 dpp ^b	20	120	2	1.9	Fibrosarcoma, nasal cavity
				7.9	Hemangiosarcoma, spleen
8 dpc	100	120	0	---	
28 dpc	100	120	1	8.2	Hepatocellular adenoma
55 dpc	100	120	5	0.2	Astrocytoma, cerebrum
				1.8	Malignant lymphoma
				7.4	Hemangiosarcoma, skin
				7.6	Prostatic carcinoma
				8.4	Malignant lymphoma
2 dpp	100	120	3	0.3	Squamous cell carcinoma, oral cavity
				5.8	Ovarian carcinoma
				8.7	Mammary carcinoma
70 dpp	100	118(2) ^c	0	---	
365 dpp	100	321(9) ^c	6	5.0	Malignant lymphoma
				6.2	Malignant lymphoma
				7.5	Transitional cell carcinoma, urinary bladder
				8.5	Mammary carcinoma
				9.0	Thyroid carcinoma
				9.4	Prostatic carcinoma
Controls (all ages)	0	359(1)	1	8.4	Mammary carcinoma

a Days postcoitus.

b Days postpartum.

c (Number dead prior to irradiation or sham-irradiation).

RESULTS

As of January 31, 1978, the dogs in this experiment ranged from 4.9 to 10.1 years of age. All exposure groups and levels are represented in the youngest and oldest populations. Table II shows the current status of the experiment with respect to survival. Twelve

TABLE IV. PROBABILITY OF DEATH DUE TO NEOPLASIA IN BEAGLES EXPOSED TO WHOLE-BODY ^{60}Co GAMMA RADIATION

Exposure level (R)	Age at exposure	No. of dogs in group	No. of dogs dead due to neoplasia		Cumulative probability of death due to neoplasia	
			Through 8.7 years	Through 9.5 years	Through 8.7 years	Through 9.5 years
0	All ages	359	1	1	.0097	.0097
20	8 <i>dpc</i> ^a	120	0	--- ^c	.0000	---
	28 <i>dpc</i>	120	1	---	.0125	---
	55 <i>dpc</i>	120	1	---	.0256	---
	2 <i>dpp</i> ^b	120	2	---	.0442	---
100	8 <i>dpc</i>	120	0	--	.0000	---
	28 <i>dpc</i>	120	1	--	.0476	---
	55 <i>dpc</i>	120	5	--	.1323 ^d	---
	2 <i>dpp</i>	120	2	3	.0182	.1273 ^d
	70 <i>dpp</i>	118	0	--	.0000	---
	365 <i>dpp</i>	231	4	6	.0314	.0722 ^d

a Days *postcoitus*.

b Days *postpartum*.

c No dogs at risk through 9.5 years.

d Probability of death due to neoplasia significantly greater than for sham-irradiated controls.

dogs died between birth and their scheduled irradiation or sham-irradiation so that only 1668 dogs are included in the analysis. All the dogs scheduled for sacrifice at 5 years of age, 34 dogs scheduled for sacrifice at 8 years and 235 other dogs in the long-term study are now dead. At the point at which all of the dogs were at risk for at least 5 years, there were no significant differences in cumulative survival statistics among different irradiation groups and between irradiated and sham-irradiated dogs.

A summary of the dogs that have died with neoplasia as the major cause of death is shown in Table III. Of 20 dogs that have died or been killed due to neoplasia, 19 have been irradiated. Eleven dogs were exposed to 20 or 100 R either just prior to or just after birth while six dogs were exposed to 100 R at 1 year of age. Four of the tumors in the perinatally irradiated dogs (55 *dpc* and 2 *dpp*) developed, and were responsible for death, at less than 2 years of age. The earliest deaths were at about 3 and 4 months due to an astrocytoma which occupied more than half a cerebral hemisphere and an oral squamous cell carcinoma. The neoplasms which occurred just prior to 2 years were a malignant lymphoma and a nasal cavity fibrosarcoma. Of the 19 neoplasms causing death or resulting in euthanasia in irradiated dogs, five have been diagnosed as malignant lymphomas. These occurred as early as 1.8 years

and as late as 8.4 years of age, and have occurred in dogs irradiated at 55 *dpc* and at 1 year of age. One dog died with a histologically benign hepatocellular tumor which comprised about half of the liver volume. Two irradiated dogs and the one sham-irradiated dog died of metastatic mammary carcinomas.

Table IV shows the cumulative probability of death due to neoplasia for all experimental groups through January 31, 1978. No fatal neoplastic diseases have occurred in dogs exposed at 8 *dpc* or at 70 *dpp*. In dogs exposed to 100 R at 55 *dpc*, 2 *dpp* and 365 *dpp* the cumulative probability of death due to neoplasia was significantly higher than that in the pooled sham-irradiated controls. Dogs exposed to 20 R at 28 *dpc*, 55 *dpc* and 2 *dpp* and to 100 R at 28 *dpc* showed consistently higher probability values of death due to neoplasia but these were not statistically significant.

Many other benign and malignant neoplasms have been diagnosed at biopsy and at necropsy in both irradiated and unirradiated dogs in this study. None of these, however, have been the cause of death or euthanasia and will not be addressed in this report. Analyses of their relation to irradiation history is ongoing.

DISCUSSION

Numerous studies of long-term effects of radiation on the beagle have been published but most of these have dealt with the effects of relatively high doses of radiation or with chronic radiation exposures. A variety of internally deposited radionuclides have been shown to induce neoplasms in the target organs receiving significant radiation doses [9-11]. Most frequently these tumors are associated with radiation doses on the order of hundreds to thousands of rads. Radiation quality has been a significant factor, but tumors have been reported in dogs exposed to alpha and beta-gamma-emitting radionuclides. Neoplasms, mainly leukemias, have also been reported in beagles receiving protracted whole-body gamma irradiation [12]. In another study, adult female beagles were exposed to 100 or 300 R of whole-body X-radiation but the results of the experiment have been inconclusive with respect to induction of neoplasms other than those of the mammary gland [13].

It should be emphasized that the results reported in this paper are of a preliminary nature. More than 75% of the dogs in the experiment remain alive and range from 5 to 10 years of age, thus only a relatively few animals have been at risk for as long as 10 years. Most of the dogs are just now reaching the age at which neoplasia is increasingly frequent. While we are still relatively early in the program, the preliminary analyses point to several findings of particular interest. First, the preponderance of neoplasms which caused death or euthanasia occurred in irradiated dogs (19/20). Second, four deaths due to neoplasia occurred prior to 2 years of age in irradiated dogs. Third, 5 of 19 fatal neoplasms in irradiated dogs were of one type, malignant lymphoma. Finally, 11 of the 19 irradiated dogs to die because of neoplasia were exposed in the perinatal period to either 20 or 100 R.

Currently it appears that there is a significantly higher probability of death due to neoplasia in the irradiated population than in the sham-irradiated controls, mainly in groups of dogs exposed to 100 R (about 83 rads). The four dogs that died within 2 years of birth were all exposed

in the perinatal period, one to 20 R and three to 100 R. Estimates of the incidence of spontaneous tumors in animals suggest that individually these early tumors in our dogs might occur by chance on the order of 1 in 100-thousand to 1 in 1-million times in animals of this age [14]. Thus they are extremely rare events. To have four in a small population and only in irradiated animals suggests a strong association with the radiation insult. Malignant lymphomas are one of the more common neoplasms in dogs [14] and of themselves are not surprising findings in any population. The finding of five cases out of 19 irradiated dogs with fatal neoplasms and none in the control population, however, suggests that an association with radiation history may exist. It is of interest to note that epidemiologic studies in man suggest that the relative risk for childhood cancer and lymphoma is increased in persons exposed to prenatal diagnostic radiation [2].

The experiment reported here occupies a unique position among studies of radiation effects in the dog in that it is the only experiment which addresses the effects of single low-level exposures during both prenatal and early postnatal life. The potential importance of age at exposure to low levels of ionizing radiation in man has repeatedly been noted. One concern is the potential for carcinogenesis, both in childhood and later in life. The results in this experiment are most certainly in a preliminary stage since over 75% of the dogs remain alive, however, it does appear that they may have considerable importance in our understanding of the role of age at exposure in radiation carcinogenesis.

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EFFECTS OF CONTINUOUS GAMMA RADIATION ON THE REPRODUCTIVITY OF MICE

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Abstract

EFFECTS OF CONTINUOUS GAMMA RADIATION ON THE REPRODUCTIVITY OF MICE.

The long-term effects of radiation on the reproductivity of CF#1/Nrs mice were studied by means of continuous irradiation at a dose rate of 57×10^{-3} Gy/22h-day throughout their entire lives, namely from conception until full growth. In the irradiated females, sterility was observed in all the mice tested at 70–80 days of age, and severe histopathological damages were also found in their ovaries. At 35 days of age, only a few abnormal follicles were counted in the irradiated ovary, whereas normal age-related changes were observed in the non-irradiated controls. In the irradiated males, significant depression of fertility was detected from their small litter sizes compared with those of the controls, and the depression of testicular weight during 70–200 days of age. Their spermatogenic activity was also depressed 50–60% compared with that of the controls. These results clearly demonstrated that the deleterious effects on fertility of male and female mice were induced by the continuous irradiation throughout their whole reproductive period including the intrauterine and the growing period after birth even at a low dose rate of gamma rays.

INTRODUCTION

Numerous investigations have been reported on the effects of reproductivity in mice irradiated continuously with a low dose rate. However, the investigation of effects in mice which bred under the influence of low intensity environmental radiation throughout the entire duration of their lives has been very limited.

Muramatsu and co-workers, Searle, and Gowen and Stadler have bred and maintained mouse populations through several generations under the influence of continuous gamma rays with the range of daily dose $4.1-24.7 \times 10^{-3}$ Gy [1-4]. According to these studies, it was concluded that small amounts of deleterious effects on fertility and genetic materials were induced by such irradiation with a low dose rate, but they accumulated to the descendants of later generations.

In the assessment of the effects of continuous irradiation the most important factor is the intensity of the daily dose applied. The present study was undertaken to demonstrate the hazardous effects of continuous irradiation on the reproductivity of mice at a dose rate of 57×10^{-3} Gy/22h-day through the entire duration of their lives in relation to the histopathological changes of the gonads.

MATERIALS AND METHODS

The animals used were the CF#1/Nrs strain of mice maintained at the National Institute of Radiological Sciences. Female mice provided with vaginal plugs after mating with normal males of the same strain were divided into two groups. One group was transferred into the gamma room, and was irradiated continuously with a 6.29×10^{10} Bq ^{60}Co source at the dose rate of about 57×10^{-3} Gy/22h-day. The progenies were raised and bred under the influence of gamma radiation throughout the entire duration of their lives. The second group was maintained and bred in the control room as non-irradiated controls.

To investigate the effects of continuous irradiation on the reproductivity, fertility, fresh weight of gonads and histopathological data were examined relating to male and female progenies in both series. The fertility test consisted of placing each male or female with one female or male for 15 days. In the irradiated series, all mice were tested immediately after leaving the gamma room. For the females, the total number of oocytes were quantitatively estimated according to the methods of Zuckerman [5] and Johns and Krohn [6], as previously used by us [7]. For males, a quantitative histological analysis of testes was carried out according to the method of Chalkley [8] and Muramatsu and Tsuchiya [9].

RESULTS

Effects on fertility of progenies

At 70-80 days of age, male and female progenies in both series were crossed by the following system: (a) a control female with a control male ($\text{C}\text{f} \times \text{C}\text{m}$), (b) an irradiated female with a control male ($\text{I}\text{f} \times \text{C}\text{m}$), (c) a control female with an irradiated male ($\text{C}\text{f} \times \text{I}\text{m}$), and (d) an irradiated female with an irradiated male ($\text{I}\text{f} \times \text{I}\text{m}$). The number of pregnant females and their average litter sizes were

TABLE I. RESULTS OF FERTILITY TEST ON THE CROSSES OF THE CONTROL AND IRRADIATED PROGENIES

Cross ^b	Number of pairs	Number of pairs progeny delivered	Number of progeny (♀/♂)	Average litter size
C♀ × C♂	70	67 (95.7)	578 (248/330)	8.6 ± 0.4 ^a
I♀ × C♂	70	0 —	0 —	—
C♀ × I♂	70	61 (87.1)	410 (195/215)	6.7 ± 0.3 ^a
I♀ × I♂	70	0 —	0 —	—

^a $P < 0.01$.^b C: Control; I: Irradiated.

analysed in Table I. As indicated in Table I, no progeny was obtained from 140 crosses of irradiated females with control or irradiated males, whereas the crosses of control females with control or irradiated males produced progenies having average litter sizes of 8.6 and 6.7 respectively. The ovaries of mated control and irradiated females were histologically examined when the fertility test terminated, and no oocytes were observed in the ovaries among the irradiated series. These results indicate that permanent sterility was induced in the irradiated females. On the other hand, fertility of the irradiated males was also depressed compared with that of the controls, the difference being statistically significant ($P < 0.01$, Table I).

Histopathological findings in ovaries of progenies

Fresh ovarian weights of 463 mice from both series were examined: 171 mice of the irradiated series ranging from 35 to 400 days (the accumulated dose of 3.14–23.94 Gy), and 292 control animals ranging from 35 to 400 days of age.

In the control series, ovarian weight increased linearly from day 35 (9 mg) to a maximum at about 200 days (30–40 mg). The weight then declined gradually until 330 days of age (10–20 mg). In the irradiated series, the ovarian weight remained low (3–5 mg) throughout the whole experimental period, but it increased at about 240 days of age owing to the appearance of ovarian tumours. The difference between both series was statistically significant ($P < 0.001$).

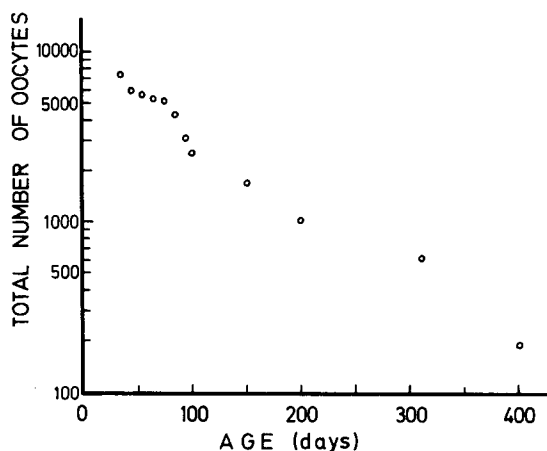


FIG. 1. Age-related changes in total number of oocytes in the control female.

Age-related changes in the total number of oocytes were observed in the control females (Fig. 1). An exponential decrease was revealed in the total number of oocytes with advancing age from 35 to 400 days, the major part of the decrease being due to the reduction of small oocytes accompanied with advancing age. In the irradiated females, the total number of oocytes was markedly decreased in all the ovaries compared with the controls of comparable age. Small oocytes had already disappeared from the ovary at 35 days of age with the accumulated dose of 3.14 Gy. At 35–65 days of age, only 6 to 2 abnormal follicles remained in the ovaries, and no oocyte was to be found in the ovaries at 70 or more days of age. This means that the ovaries were irreversibly damaged by continuous irradiation with a dose rate of 57×10^{-3} Gy/day during 55 days from the time of conception till 35 days after birth.

Histopathological findings on testes of progenies

Fresh testicular weights of 226 males in both series were examined: 109 mice of the irradiated series ranging from 65 to 200 days of age with an accumulated dose of 4.85–12.54 Gy, and 117 control animals of comparable ages (Table II).

In the irradiated series, the average testicular weight was about 50–60% of the controls, and was significantly depressed in all the age groups compared with the controls ($P < 0.05$). At 120 days of age, quantitative histological analysis of seminiferous tubules was carried out on the central cross-section of the testes

TABLE II. AVERAGE TESTICULAR WEIGHT OF THE CONTROL AND IRRADIATED PROGENIES AT VARIOUS AGES

Age (days)	Control		Irradiated		
	Number of mice	Testicular weight (mg)	Number of mice	Testicular weight (mg)	Accumulated dose (Gy)
65	11	189.7 \pm 5.1	3	113.2 \pm 3.6	4.85
70	21	172.9 \pm 4.9	26	102.5 \pm 4.4	5.13
80	36	170.3 \pm 4.5	17	124.5 \pm 3.9	5.70
100	15	191.4 \pm 7.5	14	118.8 \pm 4.1	6.84
120	19	175.9 \pm 4.7	19	91.5 \pm 2.4	7.98
170	10	193.5 \pm 5.8	14	99.4 \pm 1.6	10.83
200	6	170.2 \pm 9.2	16	104.9 \pm 5.7	12.54

obtained from 10 control and 10 irradiated males according to Chalkley's method and the method used previously by us. The contents of the testicular tissue were classified into the following categories: spermatogonia, spermatocytes, spermatids, immature spermatozoa, Sertoli cells, inner space (intratubular space), and outer space (extratubular space including interstitial cells, blood vessels, blood cells, etc.). The average proportions of each element are shown in Table III. The total quantity of spermatogenic elements was significantly depressed in the irradiated testes, and was about one-half (35.5%) of that in the control (69.5%): this reduction was a highly significant difference ($P < 0.001$). Contrary to this, an increase in non-spermatogenic elements was observed in the irradiated testes. Following this analysis, the number of type A, intermediate and type B spermatogonia, and leptotene primary spermatocytes was directly counted on 20 tubules of spermiogenic stage II–VI on each mouse in both series. In the control mice, the average number of type A, intermediate and type B spermatogonia, and primary spermatocytes per tubule was 2.95, 12.65 and 51.32 respectively. The average number of these cells in the irradiated series of mice was only one-half of that in the controls, i.e. 1.68, 6.95 and 23.47 respectively. These results reflect the depression of spermatogenic activity in the irradiated males, and account for the testicular weight depression and the smaller litter sizes.

TABLE III. AVERAGE PROPORTION OF EACH ELEMENT RELEVANT TO SPERMATOGENESIS IN THE CONTROL AND IRRADIATED MALE PROGENIES

	Control		Irradiated	
Number of mice	10		10	
Accumulated dose (Gy)	0.0		7.98	
Total number of Chalkley's counts	4000		4000	
<i>Mean frequency of each content</i>				
Spermatogonia	33.4 ± 1.9	8.4%	12.2 ± 1.7	3.1%
Spermatocytes	63.4 ± 0.7	15.9	32.3 ± 2.4	8.1
Spermatids	94.8 ± 3.3	23.7	54.9 ± 3.1	13.7
Immature spermatozoa	85.9 ± 3.3	21.5	41.5 ± 4.7	10.4
Sertoli cells	6.9 ± 1.1	1.7	28.4 ± 2.6	7.1
Inner space	77.6 ± 2.9	19.4	150.0 ± 5.0	37.5
Outer space	38.0 ± 5.0	9.5	80.7 ± 10.1	20.2
Total	400.0		400.0	

DISCUSSION

The present results suggest that the reproductive function of mice was severely damaged under the low intensity environmental radiation at a dose rate of 57×10^{-3} Gy/22h-day throughout the entire duration of life from conception until full growth.

The mouse ovary is one of the most radiosensitive organs, and is destroyed by continuous or fractionated exposures with a low dose rate even if the exposure began after sexual maturity [10]. During the infantile period until weaning the ovary is extremely radiosensitive compared with that of the adult: drastic injuries were induced by continuous irradiation at a dose rate of 57×10^{-3} Gy or more daily [7, 11–13]. The present results are in good agreement with the previous findings of Muramatsu [7]. When female mice were continuously irradiated during the developmental or postnatal suckling period with the same

dose rate as in the present study, the total number of oocytes was clearly reduced to about 60% or 0.1% of the controls through the irradiation for 20 days in utero (from conception to birth) or suckling (from birth to weaning) period, respectively (the accumulated dose was 1.14 Gy). From these results, it was concluded that the most significant effects on the irradiated ovary (induction of sterility) was mainly caused by the cell death of radiosensitive oocytes at the dictyate stage induced by the continuous irradiation during the suckling period.

On the other hand, in the male progeny, the testicular weight was severely depressed in all the ages examined, from 70–200 days old, compared with the corresponding controls. From the quantitative histological analysis of the cell population of the seminiferous tubules in these mice, it has been demonstrated that this weight depression was fundamentally caused by the decrease in spermatogenic elements. Although the mammalian seminiferous epithelium, which is a well-regulated cell renewal system, was affected by the continuous or fractionated irradiation depending upon a dose rate, spermatogenesis may be maintained under the newly formed cellular homeostatic state if the dose rate is not too high [14, 15]. In the present study, the irradiated male progeny is fertile, and normal mitotic figures of spermatogonia and their progenitor cells were frequently observed in the seminiferous epithelium with an accumulated dose of 7.98 Gy at 120 days of age. Thus it was suggested that the cell population of the seminiferous epithelium has been achieved and maintained at a near-steady state level of growth at about 50–60% of the control mice under continuous irradiation at 57×10^{-3} Gy/day throughout the entire duration of their lives.

In conclusion, the present results reveal that two major deleterious effects on fertility, namely sterility in females and depression of fertility in males, were induced in mouse populations bred under continuous gamma rays at a dose rate of 57×10^{-3} Gy/day. These effects were clearly confirmed by quantitative histopathological observations of the gonads. Further, the present results indicate that continuous irradiation up to 57×10^{-3} Gy/day for the entire duration of their lives does impair the reproduction of mice to ensure the continuation of successive generations.

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OOGENESIS, FOLLICULAR DEVELOPMENT AND REPRODUCTIVE PERFORMANCE IN THE PRENATALLY IRRADIATED BOVINE*

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Abstract

OOGENESIS, FOLLICULAR DEVELOPMENT AND REPRODUCTIVE PERFORMANCE IN THE PRENATALLY IRRADIATED BOVINE.

To determine the radioresponse of the various developmental stages of the prenatal bovine germ cell, cows bearing fetuses varying in age from 40 ± 5 (point of gonadal sex differentiation) to 270 ± 10 (approximately 13 days before parturition) days of gestation were irradiated with 300 R of ^{60}Co gamma radiation at 25–50 R/min, a level just below that likely to cause maternal deaths and gross foetal abnormalities at some developmental stages. Dose to the foetal gonad was approximately 100 rad. When the prenatally irradiated heifers were approximately 10 months old their ovaries were recovered at slaughter, serially sectioned, and prepared for microscopic analysis. A complete quantitative analysis of oocytes in primordial, growing and vesicular follicles was effected. Follicular development (reflected by counts of growing and vesicular follicles) was apparently unaffected at all ages tested ($P > 0.25$), whereas oogenesis (reflected by counts of oocytes in primordial follicles) was significantly impeded (64% of control) only between 70 and 90 days of gestation ($P < 0.05$). The germ-cell population at this period differs from later developmental stages principally by the presence of a high proportion of mitotically active oogonia, hence it appears that the oogonium is the most vulnerable cell type. Neither the viability nor reproductive performance of 60 cows that were prenatally irradiated (100 rad) at either 80 ± 10 or 130 ± 20 days of gestation was affected through a minimum of 5 years of postnatal life and three pregnancies.

INTRODUCTION

The physical and temporal attributes of the succession of developmental stages that characterize oogenesis in the prenatal human female [1] are mimicked almost precisely by the bovine [2]. It should therefore follow that what we learn about the radioresponse of the developing bovine germ cell will be valuable in our attempt to predict how the human germ cell will respond at similar developmental stages.

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The oocyte of the postpuberal long-lived mammal is particularly unresponsive to ionizing radiation whether measured in terms of oocyte survival or reproductive performance [3]. In fact, available data support the conclusion that in adult females of long-lived species sublethal exposures will not measurably affect either germ-cell survival or reproductive performance. The radioresponsiveness of cell types leading to the oocyte of the postpuberal ovary, however, is not well known. We have therefore studied the effects of acute ^{60}Co γ irradiation on the germ cells of the prenatal bovine ovary.

MATERIALS AND METHODS

Preliminary studies revealed that when administered at a high dose rate (30 R/min) an exposure of 350 R was lethal to the cow and grossly teratogenic to the fetus at certain stages. An exposure of 300 R, although sometimes lethal to the cow, resulted in a 100-rad dose to the fetus that was nonteratogenic at known critical stages. Therefore, to keep the fetal dose as high as practical an exposure of 300 R was employed, and to avoid maternal deaths the anterior two-thirds of the cows were shielded with lead plates. Cows bearing fetuses varying in age from 40 ± 5 (point of gonadal sex differentiation) to 270 ± 10 (approximately 13 days prior to parturition) days of gestation were irradiated with 300 R of ^{60}Co γ radiation at dose rates varying from 25 to 50 R/min. Dose to fetal gonads, as estimated from readings of thermoluminescent dosimeters placed in maternal bladders, was approximately 100 rad. Twenty cows were irradiated at each 10-day interval between 40 and 90 days of gestation. A like number were irradiated at each 20-day interval between days 90 and 150 and at each 30-day interval between days 150 and 270. When the prenatally irradiated heifers were approximately 10 months old their ovaries were recovered at slaughter, serially sectioned, and prepared for microscopic analysis. A complete quantitative analysis of oocytes in primordial (single layer of follicle cells), growing (two or more layers of follicle cells) and vesicular follicles was effected.

To assess the impact of prenatal irradiation on reproductive performance, an additional 140 cows were irradiated with 300 R of γ radiation when either 80 ± 10 or 130 ± 20 days pregnant. These irradiations resulted in a herd of 60 prenatally irradiated females; this herd was complemented by a group of 39 unirradiated animals. Statistical significance of treatment differences was assessed through an analysis of variance and the Student-Newman-Keuls test.

RESULTS

A dose of 100 rad of γ radiation was apparently without effect on bovine oogonia irradiated at 40 to 60 days of prenatal development (Fig. 1) when assayed at 10 months after birth. Irradiation of the day 70 oogonial population reduced the number of oocytes to 75% of control. A low of 64% occurred at day 80, and little change was seen between days 80 and 90; but the magnitude of the effect diminished greatly between days 90 and 110. Germ-cell survival increased gradually beyond day 110, and the oocyte content of ovaries irradiated at day 180 of development was at 95% of control. No significant deviations from the 180-day value occurred at either 210, 240, or 270 days of development.

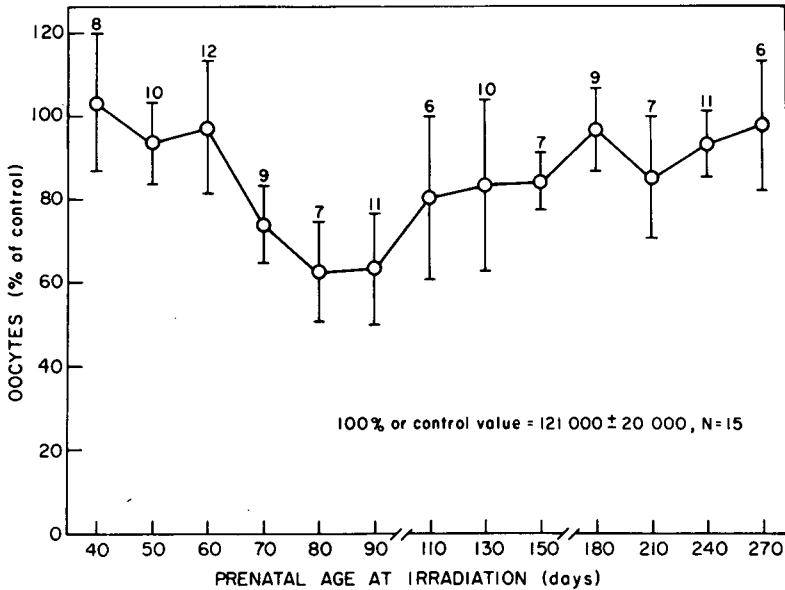


FIG.1. Oocyte population of the prenatally irradiated bovine ovary (100 rad, 6–15 rad/min). Vertical bars in this and other figures represent magnitude of standard error; number over bar is number of animals.

In no case did number of oocytes in ovaries irradiated at any single period in development differ from control at the 5% level of confidence. This level of confidence was achieved, however, when data from ovaries irradiated at days 70, 80 and 90 were pooled.

As shown in Fig. 2, prenatal irradiation apparently had no effect on follicular development, suggesting that the functional qualities of irradiated oocytes were not grossly impaired.

Results of a limited test of the effect of prenatal irradiation on reproductive performance are shown in Table I. Again, irradiation was evidently without effect.

Birth, weaning (8 months) and adult (2 years) weights are shown in Fig. 3. Average birth weights were reduced only in those calves irradiated between 40 and 80 days of gestation. Within this interval the average reduction amounted to only 13%, but when age groups were combined the difference was significant ($P < 0.01$). As evidenced by weights of the day 80 calves at 8 months (93% of control) and 2 years (89% of control), irradiation had no effect on growth rate. However, the size attained by animals irradiated prior to the 90th day of prenatal life would probably be less than control since weights of control heifers exceeded those of the day 80 heifers at birth, 8 months and 2 years. Birth and weaning weights of

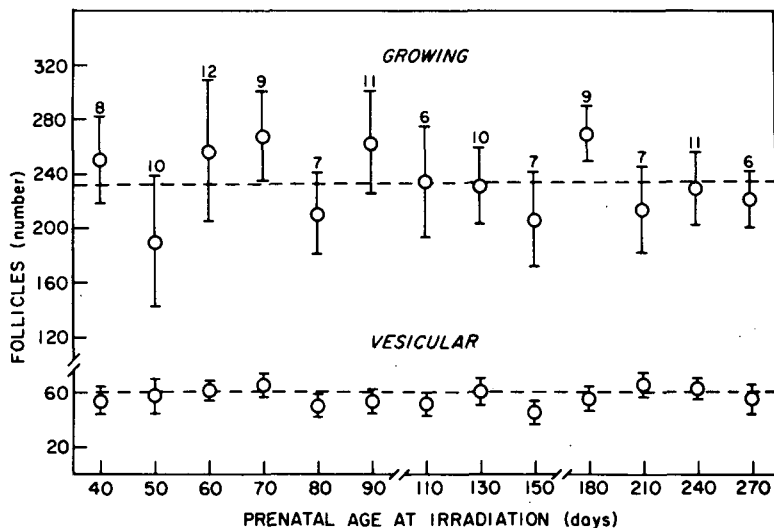


FIG. 2. Follicular content of the prenatally irradiated bovine ovary (100 rad, 6–15 min). Hatched line is control value.

TABLE I. REPRODUCTIVE PERFORMANCE OF THE PRENATALLY IRRADIATED BOVINE FEMALE (100 rad, ^{60}Co γ RADIATION)

Prenatal age at irradiation (days)	Cows* (no.)	Breedings (no.)	Pregnancies** (no.) (%)	Calves** (no.) (%)	Calves weaned** (no.) (%)
0 (control)	39	177	158 89	156 88	151 85
80 \pm 10	26	95	87 92	86 91	78 82
130 \pm 20	34	175	152 88	149 85	137 78

*Cows varied in age from 5 to 9 years and have been pregnant 3 to 7 times.

**Differences between groups are nonsignificant ($P > 0.25$).

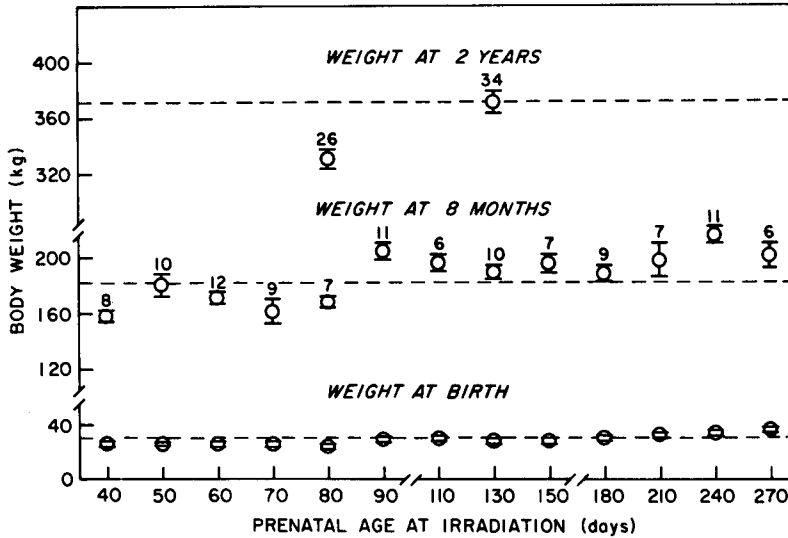


FIG. 3. Growth of the prenatally irradiated bovine female (100 rad, 6–15 min). Hatched lines are control values.

calves irradiated at prenatal ages of 90 or more days were equal to or greater than control, as were the weights of 130-day heifers at 2 years (Fig. 3). Control and irradiated populations did not differ in incidence of mortality.

DISCUSSION

The germ-cell population of the prenatal bovine female at its point of greatest vulnerability (days 70–90) is characterized by mitotically active oogonia and the initiation of meiosis [2]. The apparent absence of an effect on oogonia at earlier stages of development could be due to a combination of an inherent refractoriness in the oogonial population at those times (low mitotic rate?) and greater time between irradiation and the end of oogonial mitosis for surviving oogonia to compensate for losses. A lesser responsiveness of oogonia at early stages of development has also been observed in the mouse [4], rat [5] and chick [6]. Russell [4] noted, for example, that the reproductive performance of mice irradiated at day 9.5 of development (near the formation of the germinal ridge) was reduced to only 65% of control, while mice irradiated at 13.5 days of development (a point of active oogonial mitosis) produced only 15% of the control number of pups. As a consequence of the progressive decay of the germ-cell population that follows the cessation of oogonial mitosis, the amount of time intervening between irradiation and assay can materially influence the apparent magnitude of the effect. Beaumont [5] found in the prenatally irradiated rat that when the assay was conducted at 5 days after irradiation 97% of the control germ-cell number had survived, but when assayed at 100 days survival had apparently increased to 14% of control. Erickson [7]

also observed in the prenatally irradiated pig that the gap between irradiated and control was narrowed from 32 to 50% between 67 and 240 days after irradiation. Thus it is evident, as Beaumont [5] has noted, that in the case of a prenatally induced germ-cell deficiency the remaining germ cells are lost at less than the control rate. It is therefore likely that the extent of the effect noted in the bovine between days 70 and 90 (Fig. 1) would have been somewhat greater had the assay been conducted at a post-irradiation interval of less than 10 months.

As is the case in the human female [1], beyond day 90 in the bovine an increasing proportion of the germ-cell population becomes oocytes [2], and since the oocyte is less vulnerable to irradiation than the oogonium [5] a diminution in the irradiation effect at prenatal stages greater than 90 days would be expected (Fig. 1). Oogonial mitosis also ends in both man and bovine at approximately the 150th day of gestation, and by the 170th day essentially all oocytes have evolved to their stage of rest (diplotene, [8]), become encompassed by a single layer of follicle cells, and consequently become part of the primary or primordial follicle [2]. The LD₅₀ of the bovine primary follicle is approximately 900 rad [9], and it is therefore unlikely that sublethal doses of irradiation will have a measurable effect on germ-cell survival in fetuses older than 170 days.

As a result of this and other studies [4, 5, 6, 7], the oogonium surfaces as the most vulnerable of the germ cell's prenatal states. Available data limit reasonable interspecific comparisons to rat, pig and cow. At comparable developmental states and times of assay, germ-cell survival was reduced to 14% of control in the rat by a dose of 100 rad [5]. This same dose in the pig [7] and cow (Fig. 1) reduced survival to only 50 and 64% of control, respectively. Thus the oogonium of the long-lived mammal appears to be more resistant to irradiation than that of the short-lived mammal. The basis for this apparent difference could be inherent differences in oögonia, but a more plausible explanation is the greater time allotted to the long-lived mammal to compensate for losses (oögonia are mitotically active in the bovine for 120 days, but in the rat only 6 days elapse between the formation of the germinal ridge and the end of oogonial mitosis).

Although the data collected thus far provide no definitive answer regarding the late effects of prenatal irradiation on reproductive performance, we have learned that the ability of the prenatally irradiated bovine female to reproduce is apparently unimpaired (Table I). This knowledge coupled with the fact that neither growth (Fig. 3) nor viability was severely affected, at least through 5-9 years, leads to the conclusion that the most probable 'late effect' of an acute dose of 100 rad or less on the prenatal bovine female may be a reduction in reproductive capacity with that effect being confined only to those irradiated between 70 and 90 days of gestation. The extent to which a 36% reduction in germ-cell number may affect the reproductive capacity of the bovine is not known, but our rodent studies [10] suggest that a germ-cell loss of that amount would diminish lifetime reproductive performance by no more than 20%. The response of the bovine oogonium to chronic irradiation, however, may be quite another matter. An acute dose of 100 rad (20 rad/min) applied to the porcine oogonial population reduced the postnatal germ-cell population to 32% of control [6], but this dose applied at the rate of 1 rad/day resulted in sterility [11]. In contrast to the pig, 1 rad/day had no detectable effect on the germ-cell population of the prenatal female rat [11]. The basis for this interspecific difference is thought to be, as noted above, the

difference in the time the respective oogonial populations are at risk, this being approximately 6 and 77 days for rat and pig, respectively [11]. Oogonia are mitotically active in human [1] and bovine [2] females for approximately 120 days; therefore, if time at risk determines the oogonium's response to continuous irradiation females of both species will be more severely affected than the pig. And, like the pig, they will be far more vulnerable to a chronic than to an acute exposure to ionizing radiation.

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**ETUDE CHEZ LE RAT ADULTE DES
CONSEQUENCES D'UNE IRRADIATION
DE 150 RAD A DIFFERENTS STADES
DE LA GESTATION ET DE
LA PERIODE NEO-NATALE**
**Effets sur le développement
des organes génitaux**

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Abstract—Résumé

STUDY IN ADULT RATS OF THE EFFECTS OF IRRADIATION WITH 150 RADS AT DIFFERENT STAGES DURING GESTATION AND THE NEONATAL PERIOD: EFFECT ON THE DEVELOPMENT OF THE GENITAL ORGANS.

Gestating females were irradiated with a dose of 150 rads at a stage of gestation between ten days and parturition. Newly born animals were also irradiated during one of the first six days of post-natal life. The effects of this irradiation were then studied in adult male rats. The weight of the testicles and their histological structure, the weight of the epididymis and of the seminal vesicles as well as the plasma concentration of testosterone were determined in the irradiated animals, and were compared with those of pseudo-irradiated control animals. The absolute and relative weight of the testicles is slightly reduced after irradiation on the 15th day of gestation. The reduction is more pronounced after irradiation at later stages, and is particularly strong after irradiation between the 18th day of gestation and the third day after birth. Histological studies of the testicles of irradiated animals showed a reduction in the number of spermatogenic cells in the seminiferous tubes proportional to the atrophy of the organ. The weight of the epididymis follows the same curve as that of the testicle except for the 17th-day stage. The secretion of testosterone by the testicles of irradiated rats seems to be the same as that in the pseudo-irradiated animals. The absolute and relative reduction in weight of the seminal vesicles in irradiated adults is fairly moderate; it could be the result of a hormone deficiency during growth. Whatever the effects of irradiation may be at the cellular level in these glands, their

endocrine activity seems to be normal, and this suggests a degree of radiation resistance or rapid recovery of the secretory cells.

ETUDE CHEZ LE RAT ADULTE DES CONSEQUENCES D'UNE IRRADIATION DE 150 RAD A DIFFERENTS STADES DE LA GESTATION ET DE LA PERIODE NEO-NATALE: EFFETS SUR LE DEVELOPPEMENT DES ORGANES GENITAUX.

Des rates gestantes sont irradiées à la dose de 150 rad à l'un des stades de la gestation, entre dix jours et la parturition, les nouveau-nés le sont durant l'un des six premiers jours de la vie postnatale. Les effets de cette irradiation sont étudiés à l'âge adulte chez les rats mâles. Le poids des testicules et leur structure histologique, le poids de l'épididyme et des vésicules séminales, ainsi que le taux plasmatique de testostérone ont été déterminés chez ces animaux irradiés et comparés à ceux de témoins pseudo-irradiés. Le poids absolu et relatif du testicule est légèrement réduit après irradiation à 15 jours de gestation. Cette réduction est plus prononcée après irradiation aux stades suivants; elle est particulièrement forte après irradiation entre le 18^e jour de gestation et le 3^e jour postnatal. L'étude histologique des testicules d'animaux irradiés montre dans les tubes séminifères un dépeuplement des cellules de la lignée spermatogénétique, proportionnel à l'atrophie de l'organe. Le poids de l'épididyme suit le même profil que celui du testicule, sauf pour le stade de 17 jours. La sécrétion de testostérone par les testicules des rats irradiés ne semble pas différente de celle des pseudo-irradiés. La réduction du poids, absolu et relatif, des vésicules séminales de l'adulte irradié est assez discrète; elle pourrait être la conséquence d'une déficience hormonale en cours de croissance. Quelle que soit l'atteinte cellulaire de ces glandes par l'irradiation, leur activité endocrine semble normale, ce qui suggère une radio-résistance ou une radiorestauration rapide des cellules sécrétrices.

INTRODUCTION

L'irradiation foetale provoque une mortalité in utero, des malformations congénitales et des altérations qui se traduiront par des troubles fonctionnels lors de la vie postnatale. Ces effets dépendent de la dose de rayonnement absorbé et du stade de la gestation [1, 2].

Les conséquences de l'irradiation ont été étudiées sur les trois phases de la vie intra-utérine: préorganogenèse, grande organogenèse et croissance foetale. Notre étude est particulièrement consacrée à cette dernière période, la moins explorée dans la littérature.

Parmi les organes affectés par l'irradiation, le testicule présente une importante variation de radiosensibilité au cours de son développement foetal et néonatal [2-6]. Notre but est de déterminer la radiosensibilité du testicule de rat soumis à une irradiation de 150 rad à différents stades de la gestation et de comparer les effets sur la lignée germinale et sur la fonction endocrine.

Les lésions des cellules germinales s'accompagnent de variations de poids du testicule et de l'épididyme et de modifications de l'aspect histologique de la glande. La relation entre poids du testicule et destruction sélective des cellules germinales a été préalablement prouvée [7], et Beaumont [8] a démontré que,

pour une même dose d'irradiation, entre 13 et 19 jours de gestation, il existait une corrélation entre le poids du testicule et la proportion de tubes séminifères normaux ou stériles.

L'activité endocrine des testicules est appréciée à la fois par le poids des vésicules séminales, organes cibles de la testostérone circulante et par le dosage de celle-ci dans le plasma.

MATERIEL ET METHODES

Matériel

L'étude porte sur 400 rats mâles Sprague-Dawley provenant d'une centaine de portées. Les animaux reçoivent de la nourriture et de l'eau ad libitum. L'animalerie est éclairée de 7 h à 19 h; la température est maintenue constante à 25°C.

Détermination de l'âge des foetus

Les mâles sont mis dans les cages des femelles le soir et retirés le lendemain matin. L'âge des foetus est compté à partir de l'heure présumée de l'ovulation (1 h du matin), qui correspond environ au milieu de la période de la mise au mâle [9]. Les femelles gestantes sont reconnues par la présence de spermatozoïdes dans les frottis vaginaux après le retrait des mâles.

Irradiation

Les femelles pleines sont irradiées par une source de ^{60}Co (150 rad à raison de 10 rad par minute), soit pseudo-irradiées le matin entre 9 et 10 h à différents stades de la gestation, entre 10 jours et la naissance. Les rats nouveaux-nés sont irradiés ou pseudo-irradiés à 0, 1, 2, 3, 4, 5 ou 6 jours.

Les différents paramètres étudiés chez les animaux pseudo-irradiés avant ou après la naissance ne diffèrent pas significativement entre eux; ces rats sont donc confondus en un groupe témoin unique.

Prélèvements et mesures

Les animaux adultes (70 jours) sont pesés puis sacrifiés par un coup sur la tête. Le sang est prélevé à la carotide sur EDTA, centrifugé à 3000 tours/min; le plasma est conservé à -20°C. Le testicule droit, son épидидyme et l'ensemble vésicules séminales-glandes coagulantes sont pesés à 0,05 mg près. Le testicule gauche est fixé par le liquide de Bouin, inclus dans la paraffine et coupé à

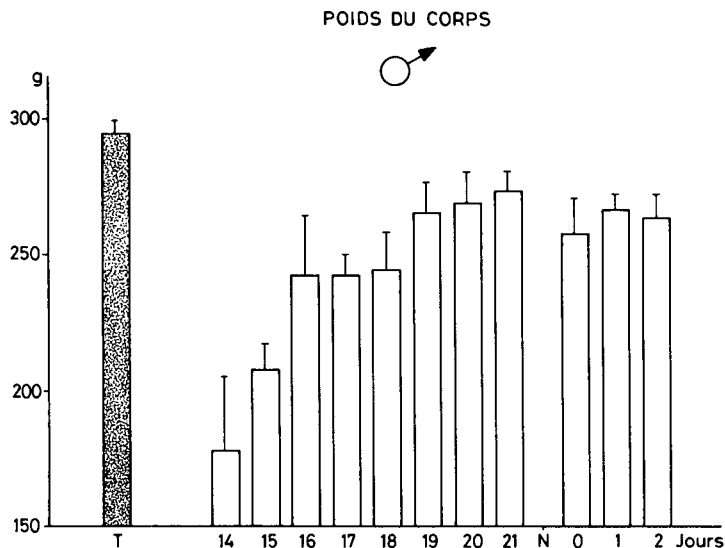


FIG. 1. Poids du corps des rats adultes mâles, témoins (T) et irradiés au cours de la gestation ou de la vie postnatale; moyenne \pm l'intervalle de confiance au niveau de probabilité $P = 0,05$; N = naissance.

5 μ m. Les coupes sont colorées par l'hémalum picro-indigo-carmin. La testostérone plasmatique est extraite par l'éther, puis sa concentration est déterminée par dosage radioimmunologique. L'anticorps utilisé pour ce dosage croise avec la dihydrotestostérone mais de façon négligeable avec les autres stéroïdes.

RESULTATS

L'irradiation foetale n'affecte pas la taille des portées, mais elle accentue la mortalité postnatale. Celle-ci est très importante aux stades précoces (100% à 11, 12 et 13 jours et 80% à 14 jours), assez faible pour les stades 15, 16, 17 et 18 jours (20%) puis normale ensuite (8%).

Poids du corps

A l'âge adulte, le poids du corps des animaux irradiés est réduit. Il l'est d'autant plus que l'irradiation au cours de la gestation est plus précoce (fig. 1). La réduction du poids représente 40% à 14 jours, 30% à 15 jours, 20% à 16, 17 et 18 jours, puis 10% environ pour les autres stades.

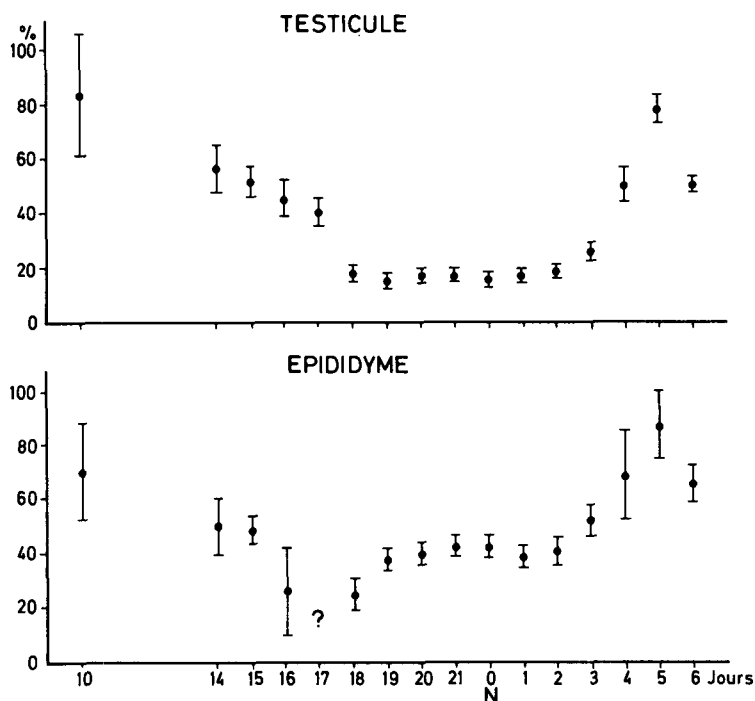


FIG.2. Pourcentage du poids du testicule droit et de son épидидyme des rats adultes irradiés à différents stades du développement périnatal par rapport à celui des organes respectifs des témoins; moyenne \pm l'intervalle de confiance au niveau de probabilité $P = 0,05$; N = naissance.

Poids et structure du testicule et poids de l'épididyme

Le poids du testicule du rat adulte est réduit après irradiation à tous les stades étudiés (fig.2). Cependant, cette réduction est plus ou moins prononcée suivant le jour de la vie foetale ou postnatale auquel a eu lieu l'irradiation.

Au cours de la gestation, cette atteinte, peu marquée à 10 jours, l'est de plus en plus de 14 à 18 jours. A partir de ce stade, et jusqu'à 2 jours de vie postnatale, elle est maximale. Cependant, du 3^e au 5^e jour, l'atrophie testiculaire devient de moins en moins importante mais présente une nouvelle augmentation à 6 jours.

Si l'on tient compte de la réduction du poids du corps des animaux irradiés, le poids relatif du testicule rapporté à 100 g de poids corporel est normal au stade 14 jours, très légèrement réduit à 15 jours, puis il suit le même profil que le poids absolu.

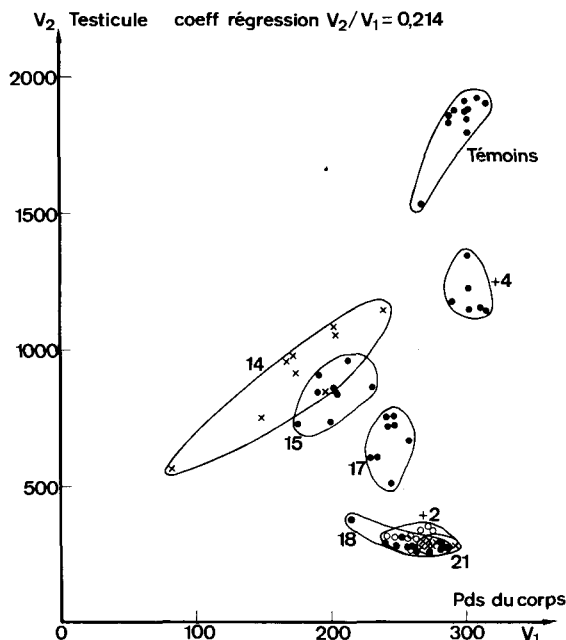


FIG.3. Variation du poids du testicule en mg (V_2) en fonction du poids du corps en g (V_1) pour les témoins et les rats adultes irradiés à différents stades de la période périnatale.

14, 15, 17, 18, 21: irradiation des foetus de 14, 15, 17, 18 et 21 jours

+2, +4 : irradiation des nouveau-nés de 2 et 4 jours.

L'influence de ces deux variables — date d'irradiation et diminution du poids du corps — est illustrée par la figure 3 à partir de laquelle se lisent deux conclusions:

- La succession ordonnée des nuages de points passe par un minimum autour de la date de la naissance du 18^e jour de gestation au 2^e jour post partum;
- la variation du poids du testicule n'est que partiellement expliquée par la variation du poids du corps (coefficient de régression = 0,214, non significatif); la sensibilité du testicule dépend donc du stade auquel est appliquée l'irradiation.

L'étude histologique des testicules montre que cette réduction du poids des testicules est liée à une abondance de sections de tubes stériles (fig.4 et 5).

L'irradiation foetale et néo-natale réduit le poids de l'épididyme de façon semblable à celui du testicule (fig.2), à deux exceptions près:

- l'irradiation des foetus de 16, 17 et 18 jours provoque une atrophie très importante de l'épididyme dont la tête est souvent réduite à quelques amas cellulaires noyés dans la graisse; au stade de 17 jours, nous n'avons pu prélever que 2 épididymes entiers (21,5% et 45%) sur les 8 cas expérimentaux;

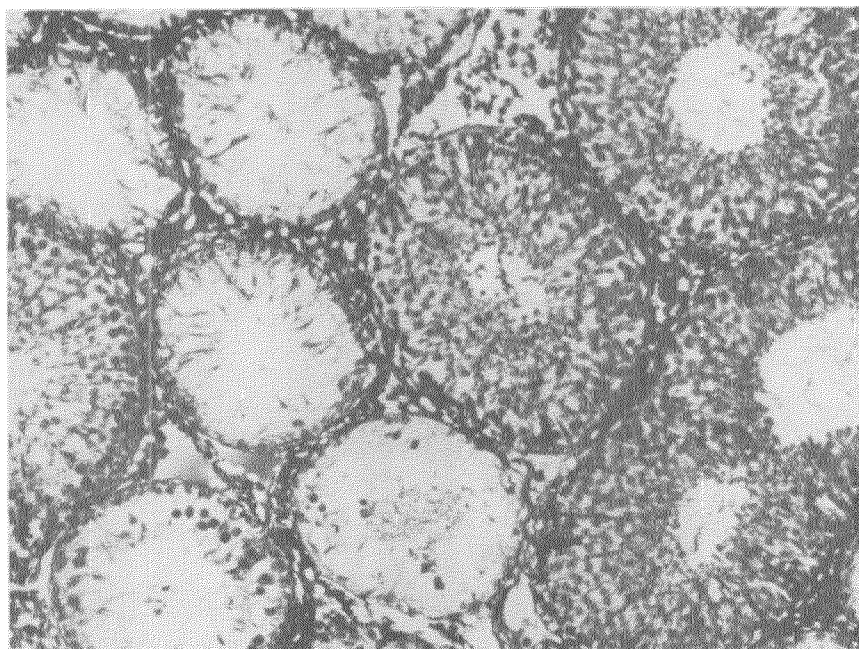


FIG.4. Section transversale d'un testicule d'un rat adulte irradié à 17 jours de gestation (X 130); on remarque la présence de tubes séminifères actifs et stériles.

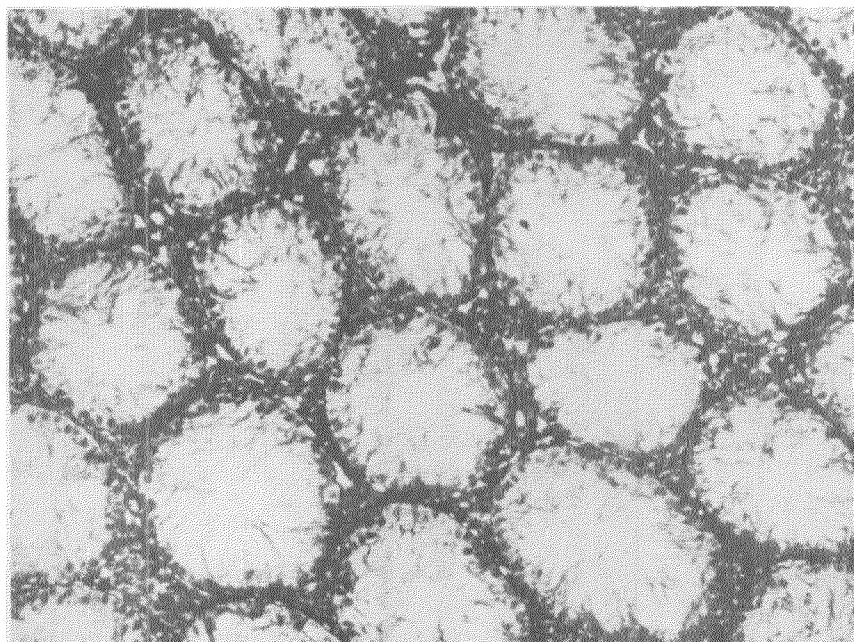


FIG.5. Section transversale d'un testicule d'un rat adulte irradié à 18 jours de gestation (X 130); on note que tous les tubes sont stériles mais que le tissu interstitiel est normal.

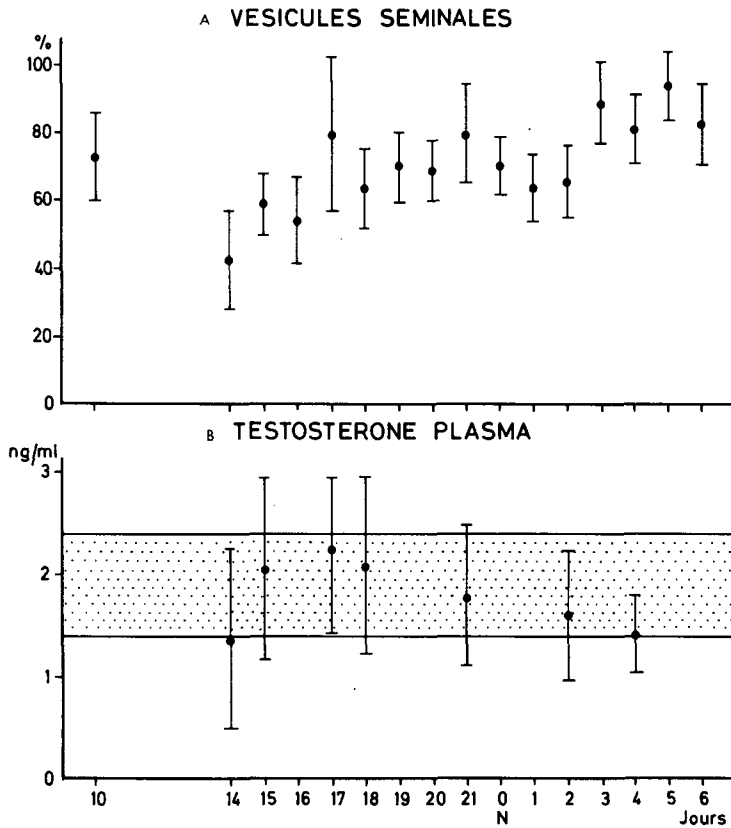


FIG.6. A) Poids de l'ensemble vésicules séminales-glandes coagulantes des rats adultes irradiés à différents stades du développement périnatal, en pourcentage de celui des témoins. B) Teneur du plasma en testostérone chez les rats adultes irradiés à différents stades du développement périnatal; la zone en pointillé représente celle où se trouvent les valeurs des animaux témoins.

Moyenne \pm l'intervalle de confiance au niveau de probabilité $P = 0,05$; $N =$ naissance.

- l'atrophie de l'épididyme est moins importante que celle du testicule pour les stades compris entre 18 jours de gestation et 3 jours de vie néo-natale (fig.2).

Poids des vésicules séminales et testostérone plasmatique

Le poids de l'ensemble vésicules séminales-glandes coagulantes est réduit après irradiation, sauf à 17 et 21 jours et au-delà du 2^e jour postnatal (fig.6A).

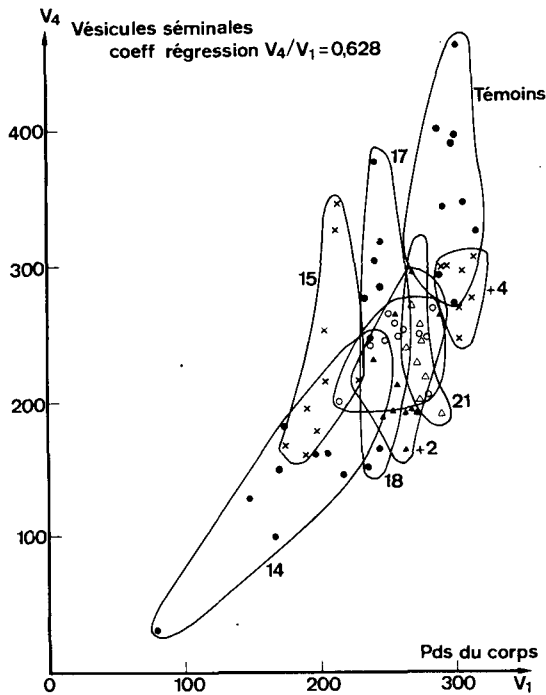


FIG. 7. Variation du poids des vésicules séminales en mg (V_4) en fonction du poids du corps en g (V_1) pour les témoins et les rats adultes irradiés à différents stades de la période périnatale.

14, 15, 17, 18, 21: irradiation des foetus de 14, 15, 17, 18 et 21 jours
+2, +4 : irradiation des nouveau-nés de 2 et 4 jours.

Mais cette réduction n'est pas significative si l'on tient compte de la perte de poids corporel des animaux irradiés. Le coefficient de régression « poids des vésicules séminales/poids du corps », égal à 0,628, est significatif (fig. 7).

Chez les rats adultes irradiés à 14, 15, 17, 18, 21 jours de gestation ou aux 2^e et 4^e jours après la naissance, la teneur du plasma en testostérone est semblable à celle des animaux témoins pseudo-irradiés (fig. 6B).

DISCUSSION

Après une irradiation de 150 rad, la radiosensibilité du foetus de 14 jours, estimée d'après la mortalité néo-natale et les effets sur la croissance pondérale de l'animal, paraît très importante mais celle de son testicule semble très faible

TABLEAU I. PRINCIPAUX EVENEMENTS DU DEVELOPPEMENT TESTICULAIRE

Age du fœtus ou du nouveau-né	Evénements et références
11 jours	
↓	Peuplement des crêtes germinales par les cellules germinales primordiales d'origine extragonadique [10]
12 jours	
13 jours	Différenciation sexuelle: formation de tubes contenant les cellules germinales [11]
14 jours	
↓	Les tubes sont bordés par une lame basale [10] Divisions actives des gonocytes [12]
18 jours	
↓	Arrêt des mitoses Phase de croissance des gonocytes situés au centre des tubes, certains dégèrent [12]
<i>Naissance</i>	
↓	
2 jours	
↓	Migration des gonocytes vers la périphérie des tubes et reprise des mitoses aboutissant à la formation des spermatogonies A [12]
6 jours	

d'après les effets sur son poids relatif. Par contre, l'irradiation du fœtus après 18 jours de gestation a peu d'effet sur la croissance pondérale de l'animal entier mais entraîne une atrophie du testicule. En fait, la radiosensibilité du testicule au cours de la période fœtale, puis néo-natale dépend du stade de développement des cellules germinales. Le tableau I en donne la chronologie.

L'irradiation de l'embryon de 10 jours ne permet pas de tuer les cellules germinales primordiales avant le peuplement des crêtes germinales. Une atrophie testiculaire a été décrite par Brent [13] après une irradiation de 100 rad à 9 jours. L'effet de l'irradiation fœtale sur le poids du testicule est peu marqué lorsque les gonocytes se divisent activement (14–17 jours). D'ailleurs, les coupes histologiques du testicule révèlent des tubes séminifères très actifs [14].

A l'opposé, les gonocytes de l'ovaire foetal qui se divisent activement durant cette période [15] sont très radiosensibles ([15] et résultats personnels). Chez les femelles, les gonocytes sont mélangés avec les cellules mésenchymateuses sans organisation alors que chez les mâles, ils sont dans des tubes [11] limités par une membrane basale et irrigués par de nombreux vaisseaux sanguins [10]. Il semble donc que l'environnement cellulaire des gonocytes mâles joue un rôle favorable pour la radiorésistance apparente de ces cellules. Cette structure organisée permettrait aux gonocytes tués dans la phase radiosensible de leur cycle mitotique d'être remplacés par les gonocytes voisins du même tube se trouvant dans une phase de radiorésistance.

La grande période de radiosensibilité des gonocytes s'étend de 18 jours de gestation à 2–3 jours après la naissance, mais certains auteurs [14] la limitent à la période foetale. Après les nombreuses mitoses (14–17 jours), les gonocytes ne se divisent plus mais s'accroissent en taille au centre des tubes. Nous formulerons quatre hypothèses pouvant expliquer cette période de grande radiosensibilité.

- In vitro, 95% des cellules de hamster chinois (lignée CHO) dont la courbe de croissance marque un plateau (arrêt des divisions) sont en phase G1 de leur cycle mitotique [16]. D'autre part, l'étude in vitro de la survie des cellules HeLa à une irradiation de 300 rad a permis de déterminer deux grandes phases de radiosensibilité au cours du cycle mitotique: l'une, la plus importante, pendant la phase M; l'autre à la fin de G1 et au début de S [17]. Si le comportement des cellules germinales foetales et néo-natales est semblable à celui des cellules CHO et HeLa, l'irradiation de 150 rad tuerait les cellules germinales toutes bloquées en fin de phase G1.

- Chez la souris adulte, Oakberg émet l'hypothèse que les spermatogonies A1 d'un fragment de tube séminifère forment un syncytium et que l'irradiation, en détruisant une cellule, tue l'association cellulaire [18]. Durant la période périnatale, il est également possible que les cellules germinales du rat forment un syncytium très radiosensible.

- Durant leur période d'arrêt mitotique, les cellules germinales situées au centre des tubes séminifères s'accroissent en taille [12]; elles sont donc dans une phase importante de synthèse protéique. Certaines de ces cellules dégénèrent spontanément [12]. L'irradiation pourrait amplifier ce phénomène.

- Enfin, l'irradiation périnatale du système nerveux central, et en particulier du complexe hypothalamo-hypophysaire est susceptible de perturber le développement gonadique. En effet, l'irradiation céphalique de 600 rads du rat nouveau-né de 2 jours provoque une importante stérilité des mâles et des modifications morphologiques sérieuses des gonades [19].

Au-delà du 3^e jour postnatal, le retour d'une certaine radiorésistance des gonocytes est contemporain de leur migration vers la périphérie des tubes et de la

reprise des mitoses aboutissant à la formation de spermatogonies de type A [12]. Nous pensons que le même phénomène de repeuplement des tubes par les cellules survivantes à l'irradiation intervient.

Cependant, la nouvelle atrophie des testicules après l'irradiation à 6 jours résulterait non seulement de la destruction des gonocytes mais également de celle des cellules de Sertoli qui se divisent alors activement [20].

L'irradiation du fœtus de 17 jours détruit l'épididyme; elle l'atteint certainement au cours d'une phase critique de son développement. Ce phénomène n'a jamais été décrit dans la littérature. L'atrophie de l'épididyme observée après irradiation aux autres stades de la gestation ou de la vie néonatale semble liée à l'absence ou la réduction du nombre de spermatozoïdes dans les voies épидидymaires.

Les vésicules séminales, organe cible de la testostérone, sont peu affectées par l'irradiation foetale ou néo-natale. Chez les rats dont le testicule est atrophié ou non par l'irradiation, la teneur du plasma en testostérone est normale. Les cellules interstitielles se différencient à partir des cellules mésenchymateuses de la gonade. Elles commencent à se différencier dès 15 jours de gestation [21] et le testicule du fœtus de 15,5 jours est déjà capable de synthétiser de la testostérone [22]. Il semble donc que les cellules interstitielles soient radio-résistantes puisque nous n'observons pas de modifications fonctionnelles. Cependant, leur fonction a pu être initialement altérée directement ou indirectement par l'irradiation foetale ou néo-natale, puis restaurée dans un deuxième temps. Ceci expliquerait la légère atrophie des vésicules séminales possédant pourtant des récepteurs hormonaux (résultats non publiés).

CONCLUSION

L'effet d'une irradiation de 150 rad du fœtus ou du rat nouveau-né dépend de l'âge de l'animal au moment de l'irradiation. Cet effet est important au début de la vie foetale en ce qui concerne la mortalité néo-natale et la croissance corporelle de l'adulte. Par contre, la radiosensibilité des cellules germinales évolue en fonction de facteurs propres liés au développement de ces cellules. La fonction endocrine des testicules n'est pas modifiée, chez l'adulte, par l'irradiation périnatale. On observe donc une dissociation très nette entre la radiosensibilité des cellules germinales et celle des cellules interstitielles.

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DISCUSSION

M. DELPLA: The testicle is one of the organs most sensitive to irradiation. Why did you apply a dose as high as 150 rads? Do you plan to resume these experiments and, if so, what dose do you intend to apply?

H.G. COFFIGNY: In an initial study on the effects of foetal and neonatal irradiation on the activity of the hypothalamo-hypophysial suprarenal axis we selected a dose compatible with a high survival rate of the animals, but which would be strong enough to disturb that axis. It was only subsequently that we became interested in the testicle. Such a dose is indeed enormous for the germinal cells at their highly sensitive stage, but a large dose is needed to have any effect on the Leydig cells. It would certainly be very interesting to study the effects of low irradiation doses on the germinal cells at their most sensitive stage.

LATE EFFECTS OF WHOLE BRAIN IRRADIATION WITHIN THE THERAPEUTIC RANGE*

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Abstract

LATE EFFECTS OF WHOLE BRAIN IRRADIATION WITHIN THE THERAPEUTIC RANGE.

Whole brain exposure with supervoltage irradiation was carried out on three sets of *Macaca mulatta*. Two sets of 12 monkeys each, at puberty, received single and fractionated exposures respectively. One set of 21 monkeys in adulthood received a fractionated exposure. Exposure to 1000 rads in a single dose, at puberty, caused no late effects. Exposure to 1500 rads caused small areas of necrosis in the forebrain white matter at 26 weeks, but a much more extensive involvement at and beyond 52 weeks that included confluent areas of necrosis in gray and white matter. Brain loss resulted in ventricular dilatation. Gliomas appeared in two out of three monkeys at or beyond 52 weeks. Exposure to 2000 rads caused such a wide scatter of focal areas of necrosis, including those in the brain stem, that survival beyond 20–26 weeks was not possible. All showed enlarged ventricular systems. Whole brain exposure, 200 rads a day, five days a week, for a course of 4000 rads, at puberty, resulted in no delayed effects. An exposure to 6000 rads, in a six weeks course, caused small, less than 1 mm, widely scattered necrotic lesions with a predilection for the forebrain white matter but not excluding the central gray matter and brain stem, at 26 weeks. Although a trend towards recession in numbers of fresh lesions was apparent, complete repair never occurred. At 52 weeks, there was considerable mineralization of the lesions and widespread telangiectasia. In the developing lesions, multiple minute breaks in the blood brain barrier caused diffuse brain swelling, reflected by papilloedema. The lesions from 8000 rads at 26 weeks were not strikingly different from those following 6000 rads. However, by 52 weeks, through an increase in number, size and coalescence, they had caused gross brain destruction. Whole brain exposure to 6000 rads in a six weeks course, in the adult, produced less effects than the same dose at puberty. The onset of the scattered necrotic lesions was

* The material in this presentation was abstracted from published [1–3] and unpublished work carried out in collaboration with T.L. Kemper, Department of Neuropathology, Harvard Medical School, Boston, Massachusetts, and S. Wakisaka and R.R. O'Neill, Laboratory of Experimental Neurology, NINCDS, National Institutes of Health, Bethesda, Maryland, United States of America.

later than expected, appearing in one out of three animals at 33 weeks, two out of three animals at 52 weeks, and two out of three at 104 weeks. The lesions at 104 weeks were predominantly mineralized, but were accompanied by a greater extent of telangiectasia than seen in the pubescent monkeys.

EXPERIMENTAL DESIGN

Therapeutic practice in recent years has included whole brain supervoltage radiation in a wide range of exposures, both in number and total amount of the radiation dose. With the intent of duplicating some of the more common present-day clinical procedures for intracranial neoplasms in man, we have undertaken three sets of observations in the monkey. The first two were carried out in animals at puberty and may be considered exploratory. In the first, three groups of monkeys were subjected to three levels of radiation in a single exposure, with animals from each group being observed sequentially for late effects. A single therapeutic dose to the whole brain is rarely employed today, but a limited number of exposures, e.g. 2000 rads in five days, is currently in use for metastatic tumors. In the second set, three groups of monkeys were subjected to three levels of radiation in fractionated exposures, that bracketed a commonly used procedure in clinical practice. These together provided a comparison between the late effects from different dose levels in a single exposure and between different dose levels in multiple exposures, in animals of the same age. Following these observations the exposure to 6000 rads in a divided dose over six weeks was employed in a set of fully adult monkeys, to more closely approximate the age of the patient as well as the therapeutic procedure now being used in the treatment of malignant gliomas in twelve University Hospitals in the United States.

MATERIALS

Twenty-four pubescent Macaca mulatta of both sexes, 3.0 kg mean weight, with an estimated age of 24 months were used for single and multiple exposures, twelve to each set. Four comparable monkeys served as controls. Twenty-one adult Macaca mulatta, male, 11.0 kg mean weight, with an estimated age of 4 to 6 years, were used for multiple exposures. All of these received 6000 rads in six weeks. Four monkeys of similar weight served as unirradiated controls.

METHODS

Single exposure, in 24-month-old monkeys.

Three groups of 4 monkeys each received 1000, 1500 and 2000 rads to the whole brain respectively. Restrained with care, the animals were fully alert during the exposure. The source of the radiation was a linear accelerator with an electron beam energy of 20 MeV, that after collimation was converted to photons by tantalum foils and flattened by a lead disk to provide a nearly constant radiation field. The

body was shielded with 3-8 inches of lead, except for an opening limited to the external borders of the brain, as determined by routine skull X-ray films. Dosimetry by thermoluminescent dosimeters and ionization chambers indicated a variation of less than 5% throughout phantom brains, unilaterally irradiated from the left side. The dose rate at midplane was 200 rads/minute. The source axis distance was 150 cm. The geometry of the exposure was determined by G. Brunhart, Ph. D., and the irradiations were carried out at the U.S. Armed Forces Radiobiology Research Institute. Prior to and at four week intervals following exposure, neurologic, funduscopic and electroencephalographic (EEG) examinations were recorded. One from each group was scheduled for sacrifice at 26, 52, 78, or 104 weeks after irradiation. When killed the brains were fixed by perfusion with paraformaldehyde, embedded whole in celloidin, and cut in serial sections at a thickness of 35 microns. Every 20th section was stained for myelin by the Loyez method and the adjacent section for nerve cells and glial nuclei with the Nissl method. Two additional sets, 100 sections apart were stained respectively with hematoxylin and eosin and periodic acid-Schiff (PAS), for vascular and other structures.

Multiple exposures, in 24-month-old monkeys.

The whole brain was subjected to a fractionated exposure of supervoltage irradiation at approximately 200 rads a day, 5 days a week, with the same equipment described for the previous series. Three groups of 4 monkeys each, received, 4000, 6000 and 8000 rads in courses of 4, 6 and 8 weeks, respectively. One from each group was scheduled for sacrifice at 26, 52, 78 and 104 weeks after the beginning of the irradiation. When killed, the brains were processed as in the previous series.

Multiple exposures, in adult monkeys.

All 21 monkeys received 6000 rads in a six week course, 200 rads a day, 5 days a week, alternate sides of the whole brain being exposed on alternate days, using the same equipment as described above. Groups of three monkeys each were sacrificed, at 8, 12, 16, 24, 32, 52 and 104 weeks after the beginning of the irradiation. The brains were perfused, embedded in celloidin, and serially sectioned for histological preparations as described for the preceding monkeys.

RESULTS

Single exposure, in 24-month-old monkeys.

1000 rads: In the four monkeys exposed to 1000 rads, there were no clinical or pathological abnormalities.

1500 rads: In contrast, those irradiated with 1500 rads showed progressive neurologic abnormalities. Monkey L-32, killed at 26 weeks, had diminished motor activity from the 24th week. L-31, killed at 52 weeks, showed myoclonic jerks of the trunk and proximal limb muscles, easily provoked by auditory stimuli, beginning at 26 weeks. There was a progressive depression in the visual evoked response (VER) beginning in the 25th week and delta activity in the background EEG from the 30th week. Optic disc pallor and behavioral loss in visual acuity was noted from the 50th week. L-30, killed at 78 weeks, showed early papilledema from the 10th through the 14th week that receded by the 18th week. The VER was depressed from the 18th week,

with minimal response from the 38th to the 50th week. High amplitude slow waves were prominent in the EEG from the 30th to 52nd week. From the 28th week, voluntary motor activity was limited, the animal exerting itself only for feeding. L-33 killed at 102 weeks, experienced a gradual reduction in VER until stabilization at low amplitude at 56 weeks. Impairment in VER latency occurred from the 94th week. Optic disc margins were blurred at the 23rd week, and at 100 weeks papilledema was pronounced. Dilated and nonreactive pupils with behavioral blindness were noted from the 100th week. Motor activity was diminished during the latter part of its course and from the 101st week, it could not stand, and had difficulty chewing food.

The gross appearance of the brains at or beyond 52 weeks revealed evidence of swelling and symmetrically dilated ventricular systems. When sectioned for histological examination, monkey L-32, killed at 26 weeks, showed scattered focal lesions in the forebrain white matter, with a predilection for the corona radiata and centrum semiovale. These consisted of discrete, minute areas of necrosis, 1 mm or less in diameter. The more recent appeared as empty or debris-filled spaces. Later lesions were in various stages of repair, with an influx of macrophages and finally mineralization. The latter were much smaller than those in the early phase (Fig. 1). When L-31 was killed at 52 weeks, widespread areas of coalescing necrosis involving primarily the white matter of the cerebral hemispheres but extending into the cerebral cortex, along with focal lesions in the brain stem and cerebellum, were evident. Although there was some mineralization in the focal lesions, little was found in the confluent areas of necrosis. Vascular changes included occasional hyperplasia in small vessels and capillary telangiectasia (Fig. 2). There were two small solid tumors in the white matter of the left occipital lobe. These were densely cellular with predominantly dark staining nuclei mixed with cells with larger, lighter staining nuclei. A single multinucleated giant cell was found. L-30, killed at 78 weeks, had a pattern of confluent focal necrosis similar in kind to but less in degree than that in L-31. There was no tumor found but adjacent to the areas of confluent necrosis there was an abnormally dense gliosis that extended some distance from the necrotic area. On gross examination, atrophic as well as edematous changes and cerebellar tonsillar herniation were seen in L-33, when the animal was necropsied at 102 weeks. Histologic examination showed prominent confluent lesions in the central gray matter and brain stem, Table 1. The most dramatic change in this brain was the appearance of three separate 'malignant' brain tumors (Fig. 3). The largest of these was centered in the mid part of the right cerebral hemisphere. From here it extended medially and ventrally to the ipsilateral thalamus. The second tumor was smaller in size with its main mass located deep in the left parietal lobe. The third tumor was in the pontine tegmentum on the left side. It extended from the upper medulla to the most caudal part of the mesencephalon. All three tumors showed a similar morphology. They were densely cellular with predominantly dark staining nuclei intermixed with cells that had larger more lightly staining nuclei. Bizarre nuclear patterns, multinucleated giant cells and mitotic figures were abundant. Central areas of necrosis were present in the two larger tumors as was vascular proliferation at their periphery. Hemorrhages within the tumor were infrequent. In all areas glial fibrils were identified with the phosphotungstic acid hematoxylin stain.

2000 rads: With whole brain exposure to 2000 rads, none of the monkeys survived beyond 26 weeks. Their deteriorating condition included crippling loss in motor power and dexterity, with finally an inability to ingest food. Three of the 4, L-35, L-37 and L-34, had pronounced papilledema beginning in the 8th week; the fourth, L-36, had blurred disc margins in the 12th week but this was less evident at the time it was killed. VER was impaired in all monkeys, beginning between the 8th and 16th week. Delta activity was pronounced in one, L-36.

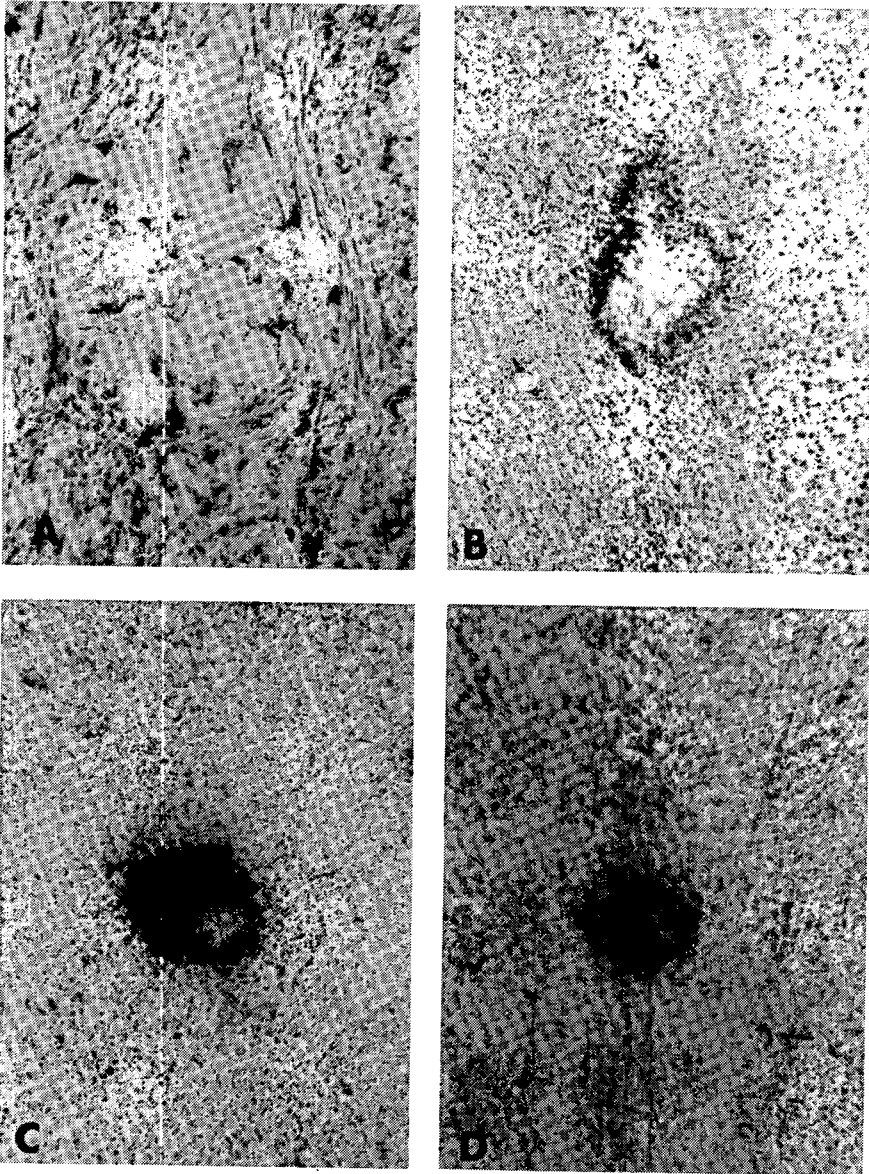


FIG.1. Photomicrographs of individual foci of necrosis. The recent lesions are relatively large and empty except for cellular debris (A). During their subsequent repair with an influx of macrophages (B) they become progressively smaller with a modest glial response, and (C) show early mineralization. Phases B and C are referred to in the text as 'intermediate'. Old lesions (D) are still smaller, and filled with deposits of calcium and iron. Nissl $\times 63$.

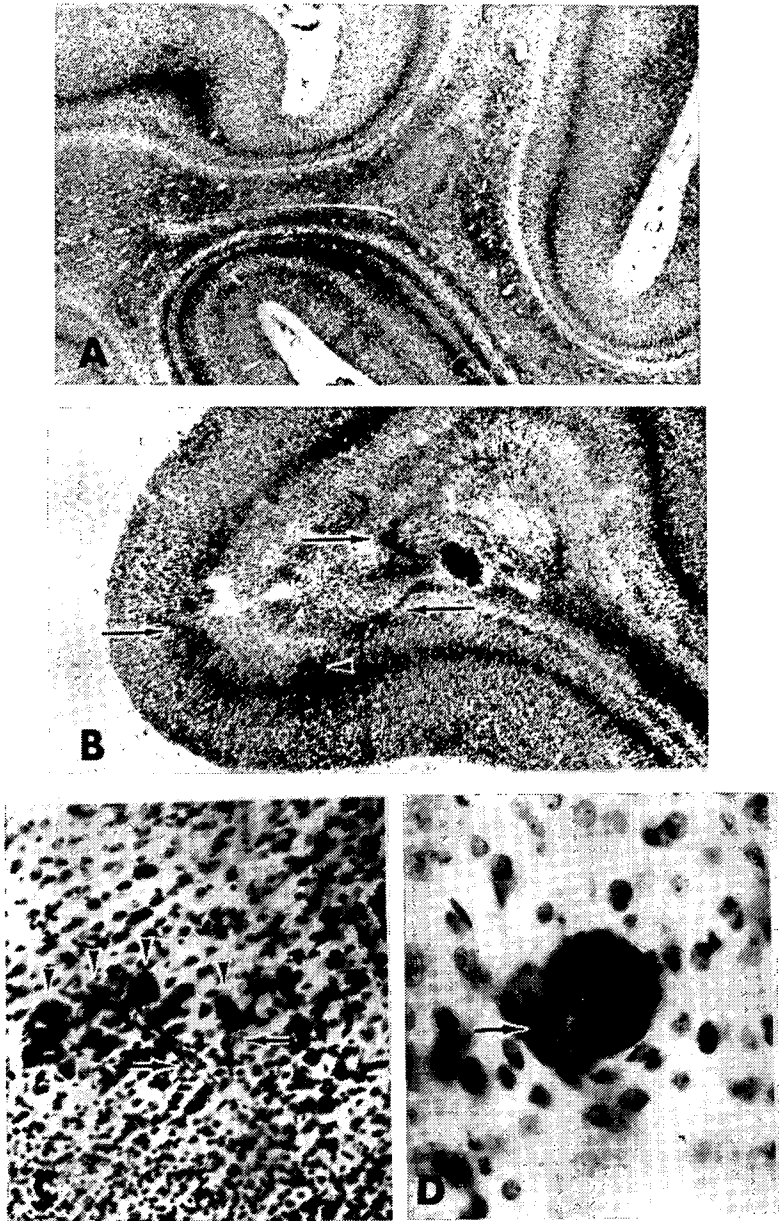


FIG.2. Vascular changes in monkeys exposed to 1500 rads. (A) is an example of telangiectasia that can occur in widely dispersed patches, without any recognizable relation to the small, necrotic lesions. Capillary proliferation, with hyperplastic endothelial cells, are indicated by the arrows in (B). They are seen to be near but not immediately adjacent to the necrotic areas. The area next to the arrow head in (B) under higher magnification in (C) and (D) shows the heaped up endothelial cell nuclei. Nissl (A) $\times 17$; (B) $\times 35$; (C) $\times 80$; and (D) $\times 400$.

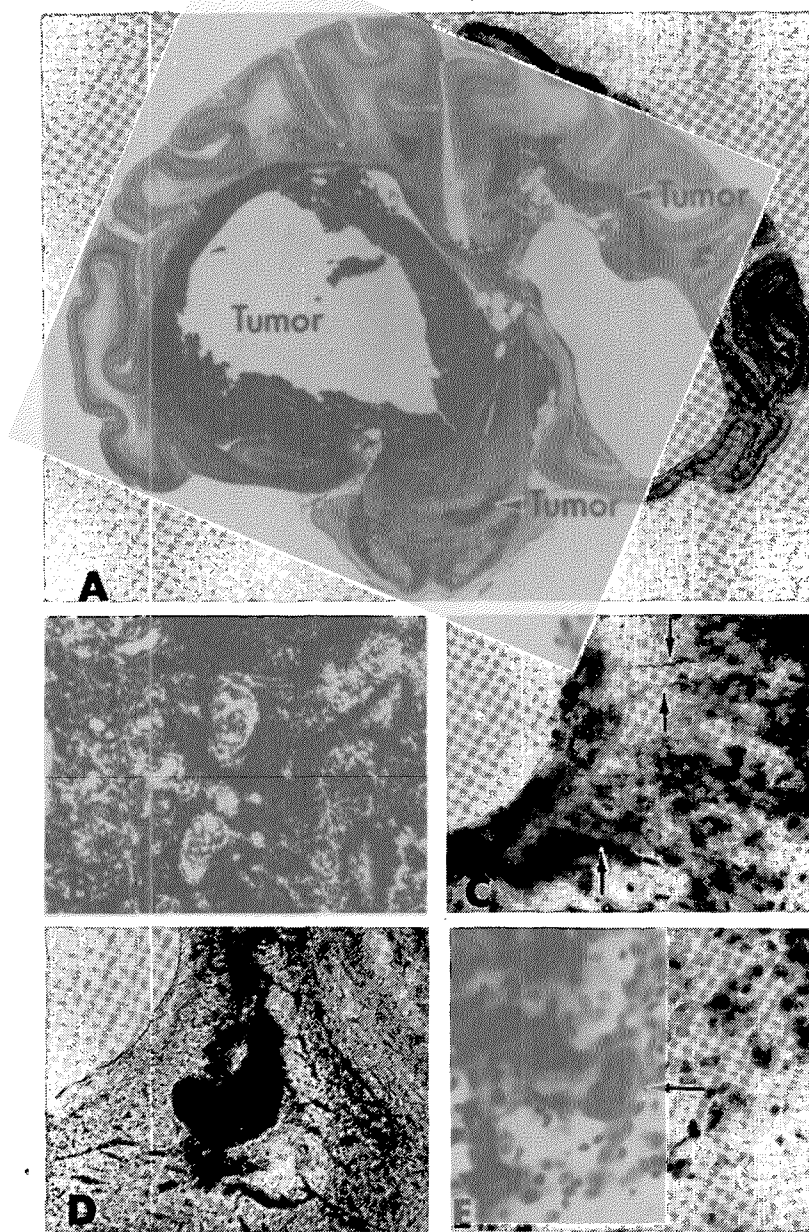


FIG.3. In monkey L-33, killed at 102 weeks after exposure to 1500 rads, there were three apparently independent glioblastomas (A). The tumors in all locations showed prominent pleomorphism of cellular and nuclear configuration, giant cells and mitosis. In (B) are seen on the left vascular proliferation in adjacent tissue and, on the right, palisading. In phosphotungstic acid hematoxylin stained sections, glial fibrils were readily identified, arrows in (C). In animal L-31, killed 52 weeks after exposure to 1500 rads, there were two small solid tumors; one of which is shown in (D). A single multi-nucleated giant cell was identified (E). Nissl (B) $\times 32$; (C) $\times 400$; (D) $\times 40$; and (E) $\times 400$.

TABLE I. DISTRIBUTION OF FOCAL LESIONS AFTER SINGLE EXPOSURE

Structure	1500 rads				2000 rads			
	26 wks	52 wks	78 wks	102 wks	19 wks	20 wks	20 wks	26 wks
	L-32	L-31	L-30	L-33	L-36	L-35	L-37	L-34
Cerebral cortex		Confluent	5	1				
Corona radiata	31	Confluent	Confluent	Confluent	303	627	122	669
Centrum semiovale	32	Confluent	Confluent	Confluent	213	406	125	446
Corpus callosum		Confluent	2	Confluent	6	67	12	55
Anterior commissure								
Fornix		4				20	10	7
Internal capsule		Confluent	18	25	65	53	21	66
Thalamus		Confluent	8	39		4	22	22
Hypothalamus		2		1	12	3	12	37
Subthalamus		Confluent		6	6	2	1	7
Optic tract					4	2	8	16
Basal ganglia		Confluent	3	16	12	1	3	15
Amygdala					1		6	
Archicortex		Confluent			4	14	16	46
Tectum		12		13	2			4
Tegmentum, mesen- cephalon		57	10	53	53	27	49	68
Tegmentum, pons		23	4	31	46	39	38	120
Basis pontis		13		4	95	95	108	150
Medulla		2	1		49	22	12	25
Cerebellar cortex								
Cerebellar white matter		52	12	7	27	79	49	73
Cerebellar roof nuclei		3	1		29		1	1
Total number of focal lesions	63	(168)	(64)	(196)	927	1 461	615	1 827

When killed, the most striking gross abnormality in the brains from monkeys L-34, L-35, L-36 and L-37, was the symmetrical enlargement of the ventricular system. The leptomeninges were unremarkable and the exit foramina were patent. Upon histological examination, all had a wide scatter of focal necrotic lesions that, though numerous and extensive, had not yet shown the coalescing or confluence that was exhibited after a longer interval (52 weeks) by monkey L-31 with the smaller exposure (1500 rads). The large number of fresh lesions in the brain stem undoubtedly contributed to the inability to survive, Table I. There were also lesions in the cranial nerves that may have interfered with masticatory movements.

Multiple exposures, in 24-month-old monkeys.

4000 rads: The four animals that received 4000 rads in four weeks showed no clinical or pathological abnormalities.

6000 rads: Of those that received 6000 rads in six weeks, the VER of monkey L-13, killed at 26 weeks, was altered from the 10th week. L-19, killed at 52 weeks, had pronounced papilledema from the 8th to the 24th week, with optic disc pallor thereafter. High amplitude slow waves in the background EEG were evident from the 24th week, as was an impairment in VER. L-14 killed off schedule, because of an unrelated, intercurrent infection, at 66 weeks had demonstrated no neurologic signs other than blurring of disc margins from the 28th week and loss of appetite from the 65th week. On the other hand, L-16, killed at 78 weeks, showed well-defined papilledema from the 8th to the 16th week, depression in VER from the 16th week, and high amplitude slow waves in the background EEG at 32 and 36 weeks.

When killed the gross appearance of the brains was not remarkable. Histologically, L-13 killed at 26 weeks, showed 143 lesions scattered throughout the forebrain, predominantly in the white matter of the corona radiata, centrum semi-ovale, and internal capsule, with a fair number in the central gray matter. These consisted of discrete areas of necrosis 1 mm or less in diameter, attended with varying degrees of macrophage reaction. The individual lesions were of different ages. The more recent consisted of pale areas, with a central core of necrotic brain surrounded by a narrow rim of macrophage response. Slightly older lesions contained numerous macrophages throughout. The oldest had mineral deposits that stained for both calcium and iron. These were quite similar in nature to the scattered minute lesions after the 1500 rads single exposure at 26 weeks (Fig. 4). Myelin sections adjacent to the Nissl sections at times revealed corresponding areas of pallor that often exceeded in extent the area of necrosis, indicating focal 'vasogenic' edema. The wide scatter of these lesions is best appreciated with whole brain serial sections. These could be missed or their extent underestimated if the brain was examined with routine blocks and thin sections. Near the punctate lesions were occasional grossly altered vascular endothelial cells. Quite apart from these were scattered areas of capillary proliferation or telangiectasia, 5-8 mm in extent.

The histology at 52 weeks was different from that at 26 weeks. L-19 had only 12 small necrotic lesions, and most of these were mineralized. In addition, there were innumerable very minute areas of mineral deposits that may have represented remnants of larger necrotic lesions, degenerated vascular walls, or plasma products. A more active process was reflected in widespread areas of dilated capillaries or telangiectasia, more than were seen at 26 weeks. L-14, killed at 66 weeks, had 63 small necrotic lesions with varying degrees of associated reactions including mineralization in 49. L-16, killed at 78 weeks showed a wide distribution of 242 focal necrotic lesions, with a greater extension into the brain stem than was

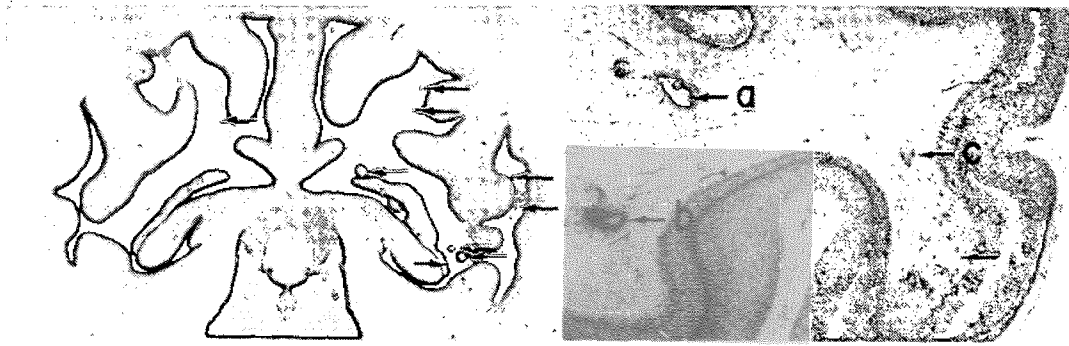


FIG. 4. Monkey L-13, killed 26 weeks after the beginning of 6000 rad course. Left: Scattered punctate lesions. Myelin $\times 1.6$. Right: Lesions of different ages in the same section; (a) and (b) are older with mineralization; (c) necrotic area filled with macrophages; (d) fresh necrosis. Nissl $\times 13$.

TABLE II. DISTRIBUTION OF FOCAL LESIONS AFTER FRACTIONATED EXPOSURE:
24-MONTH-OLD MONKEYS

Structure	6000 rads				8000 rads			
	26 wks	52 wks	66 wks	78 wks	26 wks	32 wks	52 wks	78 wks
	L-13	L-19	L-14	L-16	L-20	L-22	L-23	L-21
Cerebral cortex	2			3		3	Confluent	Confluent
Corona radiata	93	5	46	176	16	956	Confluent	Confluent
Centrum semiovale	13		13	22	19	593	Confluent	Confluent
Corpus callosum	1				1	24	Confluent	Confluent
Anterior commissure	1							
Fornix					1	35	7	
Internal capsule	14	3	3	2	5	34	Confluent	Confluent
Thalamus	11		1	26		68	Confluent	Confluent
Hypothalamus					2	14	3	
Subthalamus					1	21	6	
Optic tract	2	1				1	1	
Basal ganglia	4	1				20	Confluent	1
Amygdala						1		
Archicortex				1		19		
Tectum	1					18	11	10
Tegmentum, mesen- cephalon				3	1	84	33	36
Tegmentum, pons		2		2	6	57	21	12
Basis pontis	1			2	11	35	24	13
Medulla						21		2
Cerebellar cortex							2	16
Cerebellar white matter				5	1	80	31	
Cerebellar roof nuclei						5	10	
Total number of focal lesions	143	12	63	242	64	2 089	(149)	(90)

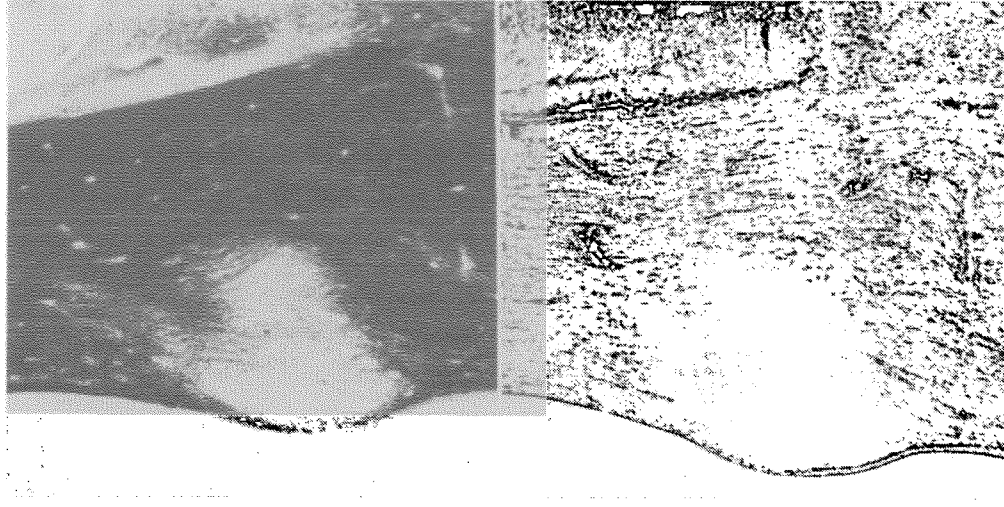


FIG.5. Monkey L-22, killed 32 weeks after the beginning of 8000 rad course, showing focal vasogenic edema in the fornix. Adjacent sections are stained with Loyez (left) and Nissl (right) X 50.

seen in the animals killed at shorter intervals. The accompanying vascular reactions and mineral deposits were, however, more apparent. From the preceding, it appears that monkeys exposed to 6000 rads have fewer fresh necrotic lesions with the passage of time and a progressively larger number are mineralized, which suggests a tendency toward healing. The monkey killed at 78 weeks had more lesions than were found at earlier intervals, but few of these were recent, sustaining the impression of a trend toward repair, Table II.

8000 rads: Of those that received 8000 rads in eight weeks, monkey L-20 killed 26 weeks after irradiation had impairment in the VER from the 8th week and in appetite and motor coordination from the 22nd week. L-22 was killed off schedule at 32 weeks because of a rapidly deteriorating condition. Papilledema was pronounced from the 6th to the 18th week. EEG delta activity was apparent from the 24th week. Becoming increasingly ataxic at 25 weeks, by 32 weeks it could not stand or sit. L-23 killed at 52 weeks had impaired VER from the 8th week, and delta activity from the 32nd. Papilledema was noted from the 8th to the 12th week, and optic atrophy from the 40th week. Pupillary response was decreased at 36 weeks and behavioral blindness was noted from the 48th week. From the 26th week, L-23 had altered motor function and by the time the monkey was killed, an incapacitating loss in strength. The VER of L-21, killed at 78 weeks, was impaired at 20 weeks, markedly depressed at 28 weeks, and exhibited no response from the 36th week. High amplitude slow waves were apparent in the background EEG from the 32nd week. Blurred optic disc margins were noted at 12 and 16 weeks, and optic disc pallor from the 40th week. Unequal pupillary size was noted periodically from the 20th week, and no response to light from the 36th week. Impaired visual acuity was suspected from the 32nd week and behavioral blindness was apparent from the 36th week. At 24 weeks, this animal became ataxic and by 40 weeks would fall when attempting to climb the sides of the cage. In the latter part of the course there were diminished motor activity and abnormal posture, with the head held in right lateral flexion.

The pathological effects from 8000 rads at 26 weeks, e.g. L-20 with 64 focal lesions, were similar in kind to those from 6000 rads, yet by 32 weeks L-22 had shown 2 089 focal lesions throughout the white and gray matter of the brain, with 115 in the brain stem. An example of focal edema in this monkey is illustrated in Fig. 5. L-23, killed at 52 weeks, demonstrated a confluence of necrotic lesions in the swollen cerebral hemispheres including thalamus and basal ganglia but fewer focal lesions in the brain stem than were found in monkey L-22 that was incapacitated at 32 weeks. L-21, killed at 78 weeks, also had massive brain destruction from confluent necrotic lesions in the cerebral hemispheres, with still fewer lesions in the brain stem than was found in L-23. The loss in brain substance was reflected in the atrophy of the gyri and marked enlargement of the lateral, third and fourth ventricles (Fig. 6). The monkeys exposed to 8000 rads demonstrate, with the passage of time, a consistent, progressive increase in number and confluence of necrotic lesions. Even with this relentless course, the monkey that was killed at 78 weeks showed a less active and more atrophic process than the monkey killed at 52 weeks.

Four control monkeys showed no functional or structural abnormalities.

Multiple exposures, in adult monkeys.

6000 rads: Unlike the variation in dose used in the 24-month-old monkeys, all 21 of the adult monkeys were exposed to 6000 rads over a six week period. They were killed at sequential intervals following the beginning of the irradiation course

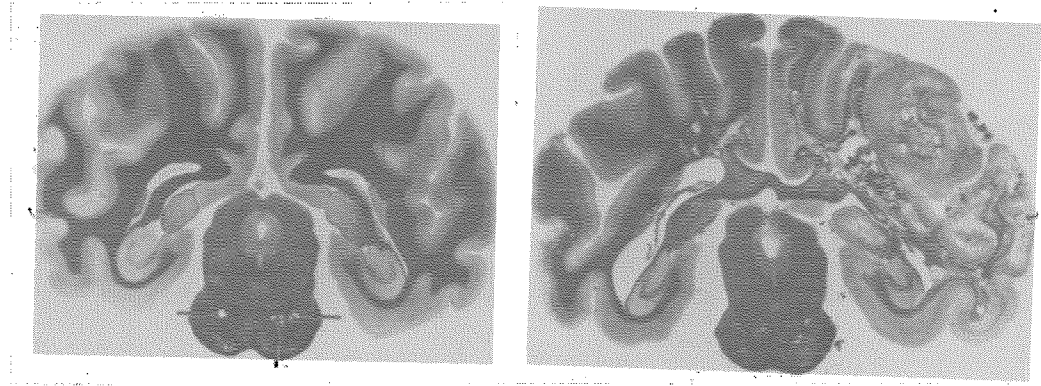


FIG.6. Left: Monkey L-20, killed at 26 weeks after the beginning of 8000 rad course, showing punctate necrotic lesions. Right: L-23, 52 weeks after start of 8000 rad course, showing confluence of lesions, gross loss in brain substance, and ventricular dilatation.

in the hope of precisely observing the evolution of the pathological lesions. Unfortunately the lack of susceptibility in the group as a whole and the delay in clinical and pathological expression were greater than expected, making our sacrifice schedule less productive than planned.

Of the twelve animals killed in groups of three at eight, twelve, sixteen and twenty-four weeks, respectively, none showed any clinical or pathological abnormality. One, LL-15, showed fundoscopic evidence of blurred disc margins between 8 and 16 weeks.

Of the three killed at thirty-three weeks, none had shown any neurologic deficit. On histological examination, one out of the three, LL-9, showed 189 small, less than 1 mm, necrotic lesions, primarily in the forebrain white matter with the largest number in the corona radiata. The degree of maturation of these lesions was predominantly intermediate, between the acute punched out defect with few macrophages and the old defect filled with mineral deposits.

Two out of the three killed at 52 weeks had shown evidence of papilledema: LL-17 between 8 and 16 weeks and LL-19 between 12 and 28 weeks. When killed, the brains of both these animals showed numerous minute necrotic lesions, 264 for LL-17 and 161 for LL-19. The greater number was in the corona radiata, but in both instances there was an extension into the cerebral cortex, 46 in LL-17 and 6 in LL-19. The degree of maturation of the lesions was predominantly intermediate, with a slight trend toward the earlier stages. Vascular abnormalities were conspicuous. These consisted of hyperplastic endothelial cells in vessels near the necrotic lesions, capillary proliferation and telangiectasia.

The three monkeys killed at 104 weeks had shown no neurologic defects. The brains of two showed scattered, minute necrotic lesions. LL-21 had 471 such defects scattered through the forebrain white matter and extending into the cerebral cortex and central gray matter (Fig. 7). Those in the white matter were all mineralized, those in the cortex and thalamus appeared as punched-out areas, with modest phagocytic activity and moderate mineral deposits. Patches of telangiectasia were widespread throughout the brain, along with occasional hyperplastic capillary endothelial cells. LL-3 had 68 lesions, all in the subcortical white matter, and all mineralized. The only other finding in this monkey was fairly widespread telangiectasia. LL-20 had only 8 lesions, all clustered in the thalamus and all mineralized, Table III.

DISCUSSION

The late biological effects of whole brain irradiation varies with the age of the host, the mode and amount of the dose and the time interval following exposure. Conceding the difficulty in interpreting these integrated factors, there are a few general conclusions that may be drawn from the described observations in the three sets of experimental animals.

1) The hallmark lesion is a minute focus of necrosis that is widely scattered throughout the forebrain white matter. These may appear as early as four or five months or as late as one to two years following the radiation.

At any given time after their appearance they are seen to be in different phases of a cycle that begins with a punched-out area, passes through phagocytosis, gliosis, and ends with mineralization. Individually, one may be in the initial phase while another is in the end phase. Larger at the outset, they are diminished in size as the cycle is completed.

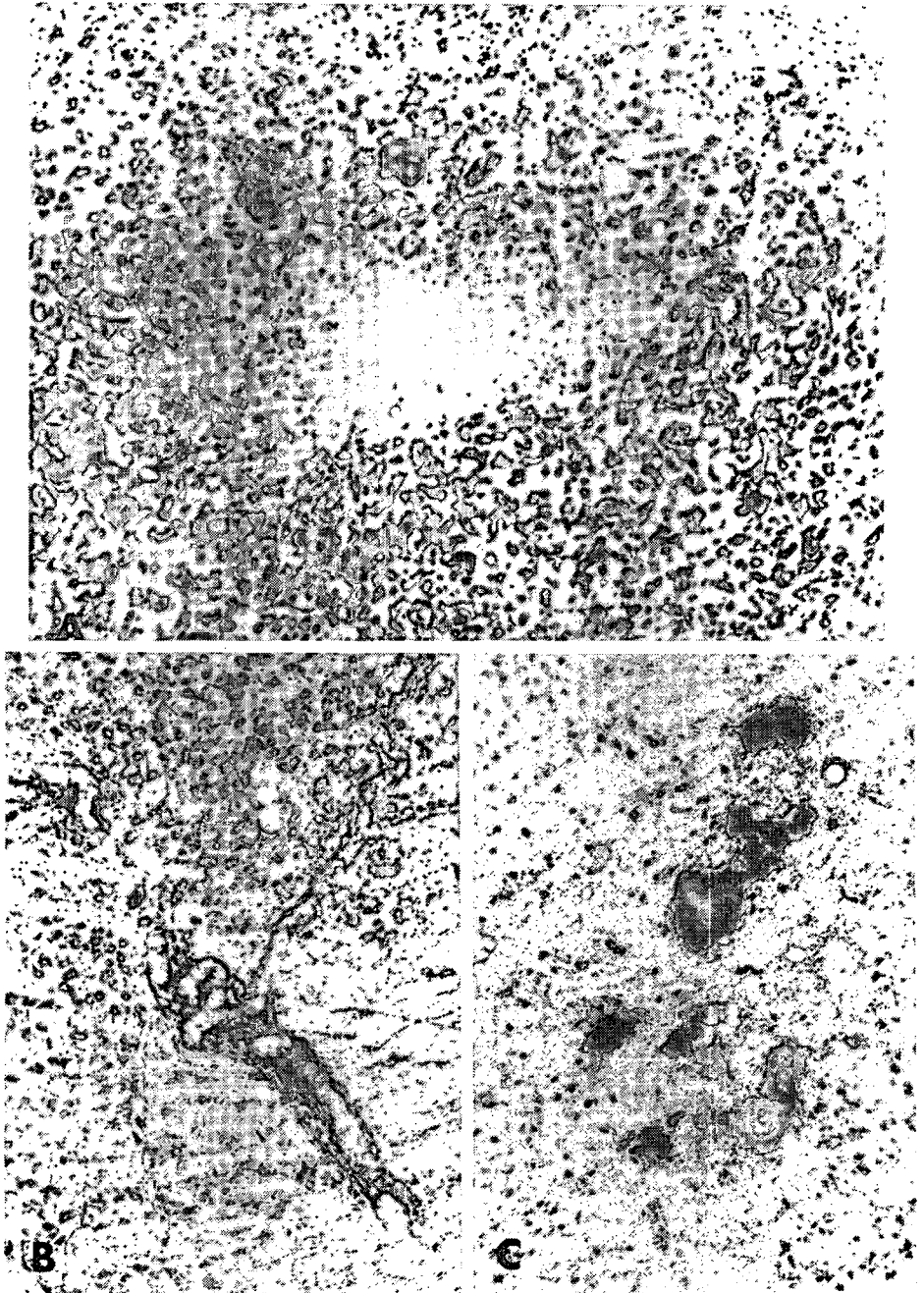


FIG. 7. (A) Adult, LL-21, 104 weeks after the beginning of 6000 rad course, showing recent lesion in cerebral cortex with adjacent capillary endothelial proliferation. (B) LL-21 showing telangiectatic vessels, remote from lesions. (C) Adult, LL-20, 104 weeks after the beginning of 6000 rad course, showing a cluster of old lesions, in the thalamus, with mineral deposits. Nissl (A) $\times 100$; (C) $\times 40$; PAS (B) $\times 63$.

In the aggregate, when there are a large number of the acute lesions, part of the effect is brain swelling from multiple minute breaks in the blood brain barrier. This is reflected in papilledema. In the aggregate, when there are a large number in the stage of mineralization, the loss in brain substance is reflected by ventricular dilatation.

2) Accompanying, or perhaps preceding the discrete areas of necrosis, are a variety of vascular abnormalities, the most notable of which are occasional absent or hyperplastic endothelial cells in adjacent capillaries. Quite apart from these are abnormal vascular channels making up patches of telangiectasia. These contribute to the brain damage and to the break in the blood brain barrier. The telangiectatic expression increased over time.

3) Whether the minute necrotic lesions proceed to a predominantly healed phase, or increase in number with confluence that results in gross brain destruction, depends both on the initial exposure and the length of time after radiation. For example, the lesions from 1500 rads in a single dose, 6000 rads in a fractionated dose and 8000 rads in a fractionated dose, look very much alike at 26 weeks, but at 52 weeks those from the 6000 rads are all but quiescent while those from the other two exposures have resulted in widespread brain destruction.

4) Malignant gliomas are a distinct rarity in the monkey [4]. However, they have been found two or more years following whole-body exposure to 600-800 rads with 55 MeV proton radiation [5]. 1500 rads of supervoltage radiation in a single dose to the whole brain at puberty is beyond any current therapeutic range, but the occurrence of neoplasms in this group, just under two years, alerts one to the possibility of such a complication.

5) The age of the host plays a significant part in the occurrence and the ultimate effect of the minute necrotic lesions. In the pubescent monkeys subjected to 6000 rads, fractionated dose, two out of four showed pronounced papilledema prior to the 24th week. Subsequent optic atrophy was seen in one of these. A third showed blurred disc margins without measurable elevation in the nerve head. By contrast, in the adult monkeys subjected to 6000 rads, fractionated dose, only two out of the twelve that were killed at or beyond 24 weeks showed definite papilledema with a third showing blurred disc margins. As in the pubescent group, these fundusoscopic changes were prior to 24 weeks.

The scattered minute necrotic lesions were seen in all four of the pubescent monkeys, killed at 26, 52, 68 and 78 weeks, respectively. By contrast the adult monkeys showed this phenomenon in only one out of three killed at 33 weeks, two out of three at 52 weeks and two out of three at 104 weeks. The third monkey in the last group showed eight lesions, all confined to the thalamus. Those at 33 and 52 weeks were predominantly in the intermediate phase, those at 104 weeks were predominantly in the late or mineralized phase. The increase in the telangiectatic expression over time was somewhat greater in the adult group than in the pubescent group.

6) Within groups of the same age with similar exposures there are individual variations in susceptibility.

7) The principal advantage in simulating a therapeutic radiation regimen in a monkey model is to observe the effects in a brain uncomplicated by pre-existing pathology, e.g. that resulting from a neoplasm, surgical trauma or chemotherapy. The principal disadvantage is that inherent in any species other than man when one wishes to interpret results in human terms. Furthermore, in irradiating the normal brain of the monkey, we are gaining no insight into the biologic variations in the human host whose brain is reacting to a growing neoplasm, surgical trauma or chemical agents. Undoubtedly, these adversely influence the effect of irradiation and may concentrate

TABLE III. DISTRIBUTION OF FOCAL LESIONS AFTER FRACTIONATED EXPOSURE: ADULT MONKEYS

Structure	6000 rads in Adult Monkeys								
	33 wks			52 wks			104 wks		
	LL-13	LL-14	LL-9	LL-24	LL-17	LL-19	LL-3	LL-20	LL-21
Cerebral cortex					46	6	3		115
Corona radiata			96	1	161	71	44		253
Centrum semiovale			67		32	66	13		69
Corpus callosum			28			5	5		8
Anterior commissure									
Fornix			2						
Internal capsule			14		18	12	3		
Thalamus			9		3			8	4
Hypothalamus									
Subthalamus									
Optic tract									
Basal ganglia									22
Amygdala									
Archicortex									
Tectum									
Tegmentum, mesen- cephalon									
Tegmentum, pons									
Basis pontis									
Medulla									
Cerebellar cortex									
Cerebellar white matter									
Cerebellar roof nuclei									
Total number of focal lesions	0	0	216	1	260	160	68	8	471

the destructive process in the region of the altered brain tissue [6]. Nevertheless, we believe it is scientifically imperative to establish a base line by studying the effects in the normal brain before proceeding to more complicated determinations.

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DISCUSSION

M. COLMAN: With reference to the fractionated irradiation experiments, were all the doses given as 200 rads per day, five days per week to the stated total doses? Also, was the radiation dose calculated at the mid-plane of the monkey brain?

W.F. CAVENESS: The answer is yes, to both questions.

J.R. MAISIN: If I understood you correctly, the lesions of the white matter are more pronounced than those observed in the grey. Have you any idea of the pathogenesis of the radionecroses observed?

W.F. CAVENESS: We believe the basic mechanism to be an abrupt focal ischaemia, based upon an impaired genetic code in selected vascular endothelial cells at the time of the exposure to ionizing radiation.

ETUDE EXPERIMENTALE DES EFFETS A LONG TERME DE L'IRRADIATION CEPHALIQUE SUR LA CIRCULATION CEREbraLE LOCALE

Résultats préliminaires

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Abstract—Résumé

EXPERIMENTAL STUDY OF THE LONG-TERM EFFECTS OF CEPHALIC IRRADIATION ON LOCAL CEREBRAL CIRCULATION: PRELIMINARY RESULTS.

The purpose of this experimental study was to follow the effects of fractionated cephalic irradiation (average dose 100 rads) on local cerebral blood circulation. Observations were made on unanaesthetized rabbits in terms of two circulatory responses, one of which is associated with rapid eye movement sleep and the other produced by inhalation of a mixture of air and 5% carbon dioxide. Both responses take the form of a characteristic increase in cerebral flow. The method of measuring variations in local cerebral flow relies on changes in the thermal conduction of cerebral tissue associated with the changes in circulation. Placement of the measuring probes entails fixation of electrodes for deriving the cortical and hippocampal electroencephalographic activity. The preliminary results refer to two animals which were subjected to three and four cephalic irradiations of 1000 rads, spaced at least a month apart, at a dose rate of $70 \text{ rad} \cdot \text{min}^{-1}$. The increase and the rate of increase of cerebral flow during rapid eye movement sleep and CO_2 inhalation proved significantly greater than the reference values from the third month on (after the second irradiation) in the case of one animal and from the sixth month on (after the third irradiation) in the case of the other. The response during rapid eye movement sleep was equal to 140% of the reference amplitude during the two observation periods in the first case, and to 110 and 150% respectively after the third and fourth irradiations in the second case. The CO_2 response was 140% of the reference value during the two observation periods in the first case, and 135% after the third and fourth irradiations in the second case. The functional significance of these changes in cerebral output is analysed in terms of the regulation of cerebral circulation.

ETUDE EXPERIMENTALE DES EFFETS A LONG TERME DE L'IRRADIATION CEPHALIQUE SUR LA CIRCULATION CEREbraLE LOCALE: RESULTATS PRELIMINAIRES.

Le but de cette étude expérimentale est de suivre l'évolution des effets de l'irradiation céphalique fractionnée à la dose moyenne de 1000 rad sur la circulation sanguine cérébrale locale. L'observation est faite sur le lapin non anesthésié, à l'aide de deux réponses circulatoires dont l'une est associée au sommeil paradoxal (SP) et l'autre provoquée par l'inhalation d'un mélange d'air et d'anhydride carbonique à 5%, toutes deux étant représentées

par une augmentation du débit cérébral, caractéristique de chaque préparation. La méthode de mesure des variations du débit cérébral local utilise les modifications de la conduction thermique du tissu cérébral liées à celles de la circulation. A la mise en place des sondes de mesure est associée la fixation d'électrodes de dérivation des activités électro-encéphalographiques corticales et hippocampiques. Les résultats préliminaires concernent deux animaux qui ont été soumis, l'un à trois, l'autre à quatre irradiations céphaliques de 1000 rad espacées d'au moins un mois, à un débit de dose de $70 \text{ rad} \cdot \text{min}^{-1}$. L'augmentation, et la pente de celle-ci, du débit cérébral, au cours du SP et de l'inhalation de CO_2 , sont plus grandes, et diffèrent significativement des valeurs de référence dès le 3^e mois pour l'un, après la 2^e irradiation, et dès le 6^e mois pour l'autre, après la 3^e irradiation. La réponse au cours du SP est, pour l'un, égale à 140% de l'amplitude de référence aux deux périodes d'observation et, pour l'autre, de 110 et de 150% respectivement après les 3^e et 4^e irradiations. La réponse du CO_2 est, pour l'un, de 140% de la valeur de référence aux deux périodes d'observation et, pour l'autre, de 135% après les 3^e et 4^e irradiations. La signification fonctionnelle de ces modifications du débit cérébral est analysée dans le cadre de la régulation de la circulation cérébrale.

1. INTRODUCTION

Les lésions du tissu cérébral apparaissant à la suite d'une irradiation locale sont connues depuis longtemps être dépendantes de la dose, du temps écoulé, de l'espèce animale et du type de cellule envisagé [1–2]. Les effets retardés apparaissent après une phase latente de plusieurs mois et peuvent évoluer sur plusieurs années.

L'atteinte cellulaire directe des cellules nerveuses serait la cause de la nécrose qui apparaît dans la substance blanche quelques mois après une irradiation de cerveau de singe [3]. Les conclusions qui en découlent sont analogues à celles qui furent faites sur l'homme [4].

L'hypothèse de l'atteinte vasculaire primitive procède, à l'origine, de la plus grande radiosensibilité de l'endothélium vasculaire [5], comparée à celle des cellules nerveuses. La nécrose du parenchyme cérébral serait la conséquence d'une ischémie provoquée par une insuffisance circulatoire locale [6–8]. Cette atteinte vasculaire pourrait également se traduire par un oedème cérébral tardif, provoqué par la rupture de la barrière hémato-encéphalique [9]. Les altérations des capillaires permettraient de penser qu'elles précèdent la nécrose des cellules nerveuses et qu'elles sont associées à celle des cellules astrocytaires [10]. Les études du métabolisme des acides nucléiques des cellules gliales montrent également que sa modification interviendrait avant la nécrose du tissu cérébral [11] et qu'elle serait secondaire à l'insuffisance capillaire [12]. Les études de la micro-angiographie réalisées sur le lapin n'ont pas permis de déterminer si les lésions vasculaires précédaient ou étaient secondaires aux lésions parenchymateuses [13].

Les études fonctionnelles s'attachant aux modifications tardives de la fonction circulatoire cérébrale après irradiation céphalique ont été conduites jusqu'à présent sur le rat [14, 15] et concluraient à une augmentation du débit

cérébral régional, bien qu'il soit observé une diminution du volume sanguin céphalique rapporté soit à l'obstruction de certains capillaires [16–18] soit à la vaso-constriction [15].

Le but de l'étude expérimentale dont on présente ici les résultats préliminaires est de suivre l'évolution des effets de l'irradiation céphalique sur la circulation sanguine cérébrale. Elle est faite sur le lapin non anesthésié à l'aide de deux réponses circulatoires dont l'une est associée au comportement de vigilance, en particulier à celui du sommeil paradoxal, et dont l'autre est provoquée par l'inhalation d'un mélange d'air et d'anhydride carbonique, réponses qui sont caractérisées par une augmentation du débit cérébral [19, 20].

L'expérimentation préliminaire a été conduite sur deux animaux qui furent soumis l'un à trois et l'autre à quatre irradiations céphaliques de 1000 rad. La limitation à deux irradiations dans l'étude en cours se place à un niveau d'irradiation pour lequel les lésions n'apparaîtraient pas chez le lapin [7, 8] et qui seraient proches du seuil de leur apparition chez l'homme [10].

2. METHODES ET TECHNIQUES

2.1. Méthode d'étude de la circulation cérébrale et de la vigilance

La méthode d'étude des effets retardés de l'irradiation céphalique consiste à mesurer les variations, au cours du temps écoulé depuis l'irradiation, de l'augmentation du débit cérébral local survenant au cours du sommeil paradoxal (SP) et de l'inhalation d'un mélange d'anhydride carbonique (CO_2) à 5% dans l'air.

Le principe de la méthode d'étude du débit cérébral local repose sur la mesure des variations du courant de chauffage d'une thermistance dont la température est élevée de quelques dixièmes de degré au-dessus de la température du tissu dans lequel elle est placée [21]. Cet index de la circulation cérébrale a été montré se rapporter au débit [22, 23] et sa fidélité a été vérifiée dans des états bien reproductibles [19]. L'observation du SP est faite à l'aide des activités électro-encéphalographiques.

2.2. Techniques

L'expérimentation utilise le lapin fauve de Bourgogne adulte, sans distinction de sexe, et de 4 kg de poids moyen.

La préparation chirurgicale est effectuée sous tranquillisation (Vetranquil (maléate d'acépromazine) 10 mg, intramusculaire) et anesthésie locale de l'animal deux mois avant l'utilisation expérimentale. Les sondes de mesure du débit cérébral local sont mises en place en région sous-corticale de l'*area precentralis* et fixées sur la boîte crânienne par un ciment dentaire. Les électrodes de dérivation des

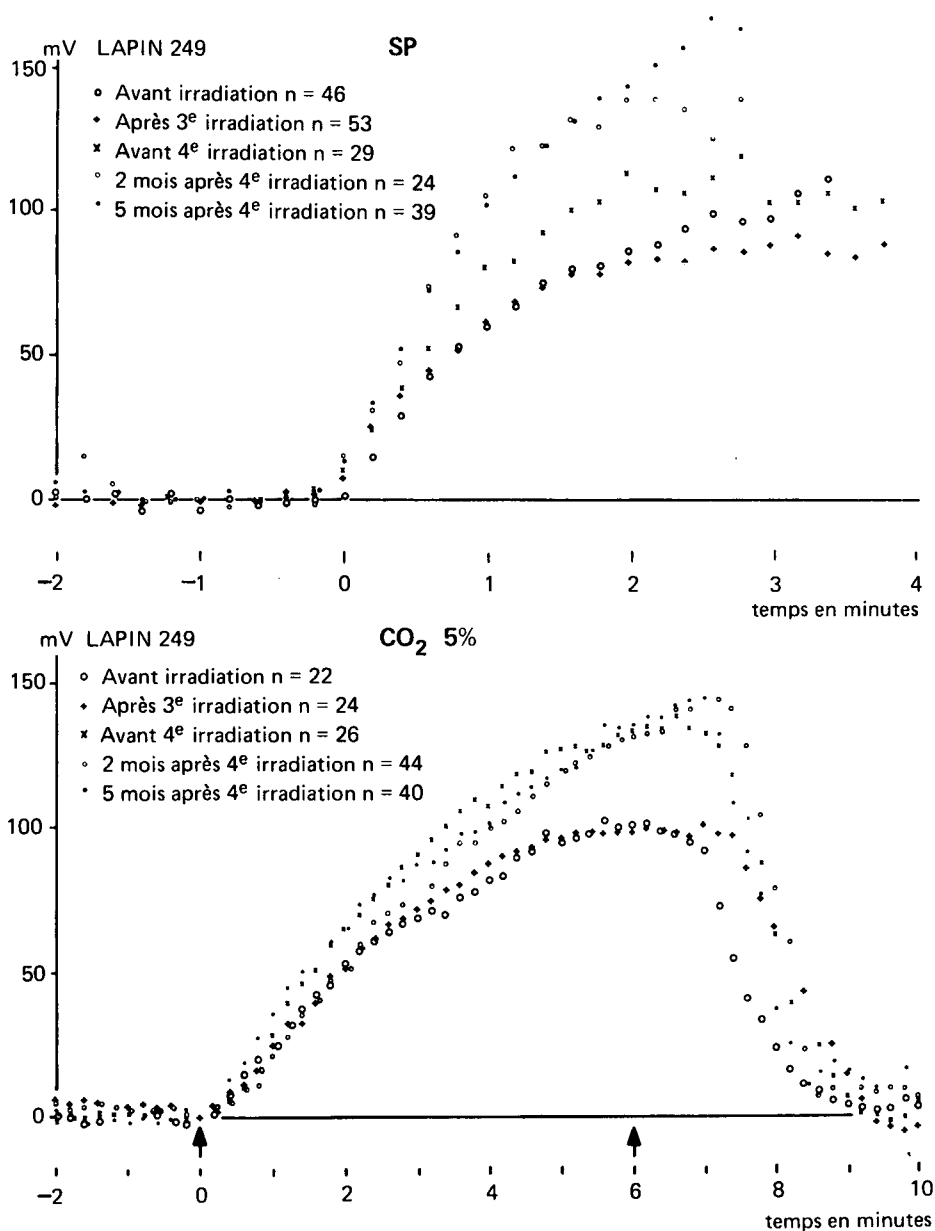


FIG.1. Le débit cérébral au cours du sommeil paradoxal (SP) et de l'inhalation d'anhydride carbonique à 5% (CO₂) avant et après irradiation.

activités électro-encéphalographiques se trouvent, pour les corticales, en regard des régions sensitivo-motrices et prémotrices. Les électrodes hippocampiques sont mises en place sous contrôle électro-encéphalographique.

L'expérimentation sur les effets retardés de l'irradiation est faite, irradiation comprise, sur l'animal non anesthésié. L'irradiation céphalique par le rayonnement γ du cobalt-60 à la dose moyenne absorbée de 1000 rad, au débit de dose de $70 \text{ rad} \cdot \text{min}^{-1}$, intéresse la région cérébrale et est réalisée en dirigeant le faisceau du rayonnement, perpendiculairement au plan sagittal, sur la région limitée en avant et en bas par l'angle extérieur de l'oeil et en arrière par le bord postérieur de la région occipitale.

3. RESULTATS

Les résultats préliminaires concernent deux animaux qui ont été soumis l'un à trois et l'autre à quatre irradiations céphaliques, espacées chacune au minimum d'un mois. Les observations après irradiation furent faites pour le premier animal (n° 248) un mois après la 2^e irradiation et deux mois après la 3^e irradiation. Elles furent faites pour le second (n° 249) 15 jours après la 2^e irradiation, 45 jours et 4 mois après la 3^e irradiation, 2 mois et 5 mois après la 4^e irradiation. La durée totale de l'observation fut de 5 mois pour le n° 248, dont 4,5 mois après la première irradiation. Elle fut de 12 mois pour le n° 249, dont 11,5 mois après la première irradiation.

L'analyse des augmentations moyennes du débit cérébral local survenant soit au cours du sommeil paradoxal, soit au cours de l'inhalation, pendant 6 min, d'anhydride carbonique à 5% dans l'air a été réalisée à chacune des périodes d'observation définies précédemment.

3.1. Le débit cérébral au cours du sommeil paradoxal

Il a été montré [19] que pour un même animal l'augmentation du débit cérébral au cours du sommeil paradoxal se reproduit fidèlement d'une séquence de sommeil à une autre. Après une phase d'augmentation de une minute et demie à deux minutes, suivant les préparations, le débit atteint un niveau qui reste stable jusqu'à la fin du sommeil paradoxal. Il est ainsi défini une pente et une amplitude maximale qui caractérisent l'augmentation du débit cérébral d'un animal déterminé.

L'amplitude de l'élévation du débit est plus grande après les irradiations réalisées chez les deux animaux. L'analyse de variance à deux niveaux, avant et après irradiation, montre une différence significative dès le 1^{er} mois succédant à la 2^e irradiation pour le premier et dès le 4^e mois suivant la 3^e irradiation pour le second (fig.1). Cette augmentation est égale à 140% de celle qui intervient

avant la première irradiation au cours des deux périodes d'observation pour le premier. Elle s'accroît avec le temps pour le second et passe de 110% après la 3^e irradiation à 155% de la valeur de référence pour les observations des 2^e et 5^e mois effectuées après la 4^e irradiation.

La pente de l'augmentation du débit s'accroît avec le temps séparant les observations de l'irradiation. La pente moyenne mesurée au cours de la première minute passe, pour le premier animal, de 90 mV à 130 mV après les irradiations. Elle passe, pour le second, de 60 mV à 75 et 100 mV après les 3^e et 4^e irradiations (fig. 1).

Le moment d'apparition du débit stabilisé est retardé après irradiation de 36 à 60 secondes. Cette observation est faite sur le n° 249, pour lequel la durée des séquences de sommeil paradoxal est suffisamment longue après irradiation pour apprécier la phase du débit stabilisé.

3.2. Le débit cérébral au cours de l'inhalation de CO₂ à 5% dans l'air

L'augmentation du débit cérébral au cours de ce test est, pour un même animal, à une période déterminée, supérieure à celle qui survient au cours du sommeil paradoxal. Son amplitude à la 6^e minute est, avant irradiation, de 160 mV pour le premier et de 100 mV pour le second (fig. 1). Après irradiation cette amplitude est augmentée chez les deux animaux. L'analyse de variance, réalisée dans les mêmes conditions que précédemment, montre une différence significative dès le 1^{er} mois après la 2^e irradiation pour le premier, et dès le 4^e mois après la 3^e irradiation pour le second. Cette augmentation, comparée à celle qui caractérise chaque animal avant irradiation, est égale à 140% pour le premier et à 135% pour le second (fig. 1); cette amplitude de l'élévation du débit n'est pas modifiée chez celui-ci après la 4^e irradiation. La moindre augmentation, observée sur le premier au 2^e mois après la 3^e irradiation, n'est pas significativement différente de la précédente, observée avant cette 3^e irradiation.

4. DISCUSSION

L'étude préliminaire qui est ici rapportée ne permet de mettre en évidence aucune manifestation d'insuffisance fonctionnelle. Les résultats expérimentaux font apparaître deux caractères essentiels aux modifications du débit cérébral qu'il est nécessaire de rattacher à la régulation de la circulation cérébrale. Il y a d'une part l'augmentation plus grande du débit au cours du sommeil paradoxal et de l'inhalation d'anhydride carbonique et, d'autre part, l'augmentation de la pente et le retard à la stabilisation du débit au cours du sommeil paradoxal.

L'augmentation de l'amplitude de la réponse du débit cérébral est à rapporter soit à un développement du lit vasculaire, et du volume sanguin correspondant,

soit à l'inhibition d'une résistance vasculaire apparue après irradiation et ayant entraîné la diminution du débit cérébral, inhibition qui ramènerait le débit à son niveau antérieurement atteint au cours des deux états étudiés. L'augmentation de la pente est suivie d'un retard à la stabilisation du débit au cours du sommeil paradoxal. La première est à rapporter à la diminution plus rapide de la résistance vasculaire cérébrale. La diminution de la pente, correspondant au retard à la stabilisation du débit, traduit une réapparition de cette résistance.

La diminution du débit cérébral fait intervenir soit une obstruction de certains capillaires par prolifération cellulaire [24] (la diminution du débit par l'augmentation de la pression du liquide céphalo-rachidien (LCR) peut être également envisagée; cette augmentation chez le singe n'interviendrait que tardivement au cours de la période d'œdème généralisé [9]), soit une vaso-constriction localisée des artères. Cette dernière hypothèse s'appuie sur le fait que l'hypertension facilite le développement des lésions cérébrales après irradiation [25, 26] et que l'hypertension entraîne la vaso-constriction localisée des artères [27, 28]. L'obstruction des capillaires ne permet pas de rendre compte d'une augmentation des réponses après irradiation sans l'augmentation de la pression de perfusion. Par contre l'hypothèse du renforcement de la tonicité des artères cérébrales rendrait compte de l'augmentation de la réponse du débit au cours de l'inhalation d'anhydride carbonique et du sommeil paradoxal par l'inhibition de cette vaso-constriction. Celle-ci, provoquée par l'irradiation, s'ajouterait à la vaso-tonicité existant avant irradiation et serait de même nature. La plus grande amplitude des réponses après irradiation traduirait en fait la suppression de cet accroissement de tonicité ayant provoqué l'abaissement du niveau de référence du débit et non une augmentation réelle du débit au cours des deux états circulatoires étudiés.

Cette inhibition de la vaso-constriction pourrait rendre compte de l'amplitude augmentée de la réponse mais est insuffisante pour rendre compte de l'augmentation de la pente sans l'intervention d'un second mécanisme qui devrait lui être associé. En aval de la vaso-constriction, la résistance vasculaire serait abaissée de façon permanente, facilitant ainsi l'augmentation de la pente dès la suppression de la vaso-tonicité. Le mécanisme de cet abaissement de résistance concourant à l'autorégulation, définie par le maintien d'un débit stable face aux modifications de la pression de perfusion, reste hypothétique.

Le schéma analogique de la régulation de la circulation cérébrale, largement accepté, la compare à deux résistances en série [29] qui représenteraient, d'une part les vaisseaux extraparenchymateux influencés par le système nerveux autonome, et d'autre part les vaisseaux intraparenchymateux dont la régulation serait assurée surtout par des mécanismes intrinsèques, d'origine métabolique ou myogénique. Nos observations antérieures ont montré [19] que l'augmentation du débit au cours du sommeil paradoxal était davantage en accord avec l'hypothèse d'une régulation de la vaso-tonicité d'origine neurogène. Une hypothèse de même

nature a été également avancée pour rendre compte de l'action de l'anhydride carbonique [30]. Cette régulation par le système autonome a fait l'objet de travaux récents [31–34] qui la confirment.

La circulation cérébrale, dans l'hypothèse de sa diminution, présenterait, quelques mois après irradiation, un renforcement de la vaso-tonicité de certains de ses éléments vasculaires. Cette vaso-constriction prédominante sur les vaisseaux extraparenchymateux provoquerait une diminution de la pression de perfusion entraînant une réaction de vaso-dilatation, par autorégulation, des éléments vasculaires intraparenchymateux non soumis ou peu soumis au contrôle neurogène. Un état circulatoire cérébral de ce type a été décrit dans le choc hémorragique du babouin [29].

L'augmentation du débit cérébral fait intervenir un développement du volume sanguin circulant. Le relâchement de la vaso-tonicité au cours du sommeil paradoxal et de l'inhalation de CO_2 associé à cette augmentation du volume sanguin peuvent rendre compte de l'amplitude et de la pente plus grandes de la réponse du débit. L'irradiation provoquerait au niveau du parenchyme une vaso-dilatation d'origine métabolique ou une augmentation du volume sanguin par néo-vascularisation.

Les modifications circulatoires envisagées s'opposent aux observations microscopiques des lésions vasculaires. Celles-ci accompagnent la nécrose des éléments nerveux [9, 10, 13, 18] ou lui succèdent [3]. S'il est envisagé une multiplication des cellules musculaires lisses [18], certains auteurs ont décrit un épaissement des artérioles correspondant à la sclérose [6, 8]. La mise en évidence au bout de quelques mois d'un exsudat plasmatique traduisant une inflammation chronique [9] pourrait être un argument à l'origine métabolique de la vaso-dilatation intraparenchymateuse envisagée.

Les études tendant à montrer une modification des médiateurs chimiques du système nerveux autonome sont encore débutantes [2] et ne peuvent apporter aucun élément favorable aux modifications de vaso-motricité envisagées.

En conclusion à cette étude préliminaire, certains points semblent acquis sous réserve de vérifications actuellement en cours. La vaso-tonicité cérébrale, dans ses rapports avec le sommeil paradoxal et l'anhydride carbonique, pour lesquels sa composante neurogénique est vraisemblable, ne serait pas modifiée ou serait même augmentée. La vaso-tonicité d'origine intrinsèque, myogénique ou métabolique, serait toujours abaissée. Les modifications du niveau de débit cérébral local restent pour l'instant hypothétiques, les deux variations opposées ayant rendu compte, au cours de cette analyse, des résultats préliminaires.

La prolongation des observations jusqu'à deux années après irradiation, en association avec la vigilance, doit permettre de suivre une évolution qui, pour l'instant, n'apparaît pas contradictoire au regard de certains résultats [15], et d'analyser au mieux les modifications comportementales et les altérations électro-encéphalographiques en relation avec celles du métabolisme cérébral.

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LATE RESPONSE OF THE CEREBRAL CIRCULATION TO X-IRRADIATION OF THE BRAIN IN THE RAT*

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Abstract

LATE RESPONSE OF THE CEREBRAL CIRCULATION TO X-IRRADIATION OF THE BRAIN IN THE RAT.

Variations in the cerebral blood volume (CBV), cerebral blood flow (CBF), circulatory mean transit time (\bar{t}) and the cerebral distribution space of iodoantipyrine (CDS) during the survival time of rats irradiated on the brain were evaluated by the use of a non-invasive radio-tracer technique. The principle of this technique is the comparison of the cephalic behaviour of two radiotracers ($^{99}\text{Tc}^{\text{m}}$ -pertechnetate and ^{131}I -iodoantipyrine) which are only clearly discriminated by the brain. The cerebral circulatory parameters are calculated from the data extracted by computer analysis from the cephalic blood dilution curves of both tracers. These curves were recorded by external counting over the head after a sudden injection of the radiotracers into the femoral vein. Repeated determinations of cerebral circulatory parameters of individual rats were performed at 6-month intervals until 18 months after X-irradiation of the brain at 2000 rads. At 6 months after exposure, CBV, CBF and CDS were not significantly modified in irradiated animals. At 12 months the value of CBF is twice that of the non-irradiated animals. However, the corresponding increase in CBV is less important. At the same time CDS is significantly reduced. At 18 months, there is no further significant modification of CBV and CBF, although CDS is relatively lower than at 12 months. These results indicate that in the absence of degenerative and occlusive change in the vessels, the failure of the brain homeostasis reflected by the CDS reduction is balanced by the increase in CBF and CBV. This suggests that the hypoxia of the nervous tissues could be an important component of radiation late effects in the brain.

INTRODUCTION

According to Rubin and Casarett [1], the inverse relationship observed in irradiated brain between the radiation dose and the delay in the development of radio-necrosis is much more compatible with a pathogenesis of radio-necrosis of

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mature nervous tissue, in which progressive vascular damage plays a primary role, than with a pathogenesis in which the primary role is played by direct damage to nervous tissue cells. Nevertheless, other authors [2] are convinced that the radio-necrosis of the brain tissue is the result of direct destructive action on the nervous tissue cells, rather than secondary to vascular damage. This latter opinion is based on the difficulty of relating the necrosis occurring after irradiation to the observed changes in the blood vessels. From a review of the relevant literature the conclusion arises that these opposite hypotheses are only justified by morphological criteria, and thus fail to take into consideration the fate of blood circulation in the irradiated brain. For this reason, in order to find circulatory arguments supporting or not the relevance of brain radio-necrosis to vascular damage, we have performed sequential investigations of the cerebral nutritive blood flow of rats irradiated on the brain.

MATERIALS AND METHOD

Three-month-old female F_1 hybrid rats of a standard strain were used. All irradiations were carried out under anaesthesia, obtained by an intraperitoneal injection of 0.025 mg of 2,2,2 tribrom-aethanol per 100 g of body weight. Animals were held in a standard jig and irradiated laterally on the brain through the side of the skull using a 25 X 15 mm irradiation field. To ensure a homogeneous distribution of the dose in the brain, each side was similarly exposed and received half the dose. We used an X-ray generator operating at 250 kV and 25 mA. The half value layer was 2 mm Cu. A total dose of 2000 rads (evaluated at the mid-brain) was delivered to four groups of 20 rats, and four groups of 20 control animals were submitted to a sham irradiation. The effect on the cerebral circulation was evaluated at less than 1 month, 6, 12 and 18 months after exposure.

Measurements of brain circulatory parameters were performed by the use of a non-invasive dilution method. The essential of this method is the study of the cephalic random walk of $^{99}\text{Tc}^{\text{m}}$ -pertechnetate (Tc) and ^{131}I -iodoantipyrine (IAP), which differ essentially in their capacity to cross the blood brain barrier. From our experimental data, it appears that the cerebral blood volume (CBV) is strongly correlated with the amount of Tc that does not leave the blood during its first passage in the head. It is calculated by the difference between the head tracer pools of IAP (HTP_{IAP}) and Tc (HTP_{Tc}) as defined by their first dilution curve. On the other hand, the difference between the cephalic fractional uptake of Tc (HTF_{Tc}) and IAP (HTF_{IAP}) at their entrance in the head, governs the variation of the cerebral blood flow index (CBF).

Furthermore, the difference between the IAP and Tc relative content in the head at equilibrium gives an estimate of the cerebral distribution space of IAP in the brain (CDS). This index is also indicative of the extent of the capillary surface

TABLE I. CARDIAC INDEX AND CEREBRAL CIRCULATORY PARAMETERS IN RATS BREATHING ROOM AIR

Animal No.	CI (ml/min per kg)	CBF (ml/s per g)	CBV (ml/g)	CDS (g)	\bar{t} (s)
Rat 1	222	0.0095	0.0126	1.1567	1.326
2	223	0.0063	0.0088	1.1185	1.397
3	250	0.0152	0.0155	0.9417	1.020
4	300	0.0093	0.0071	0.7050	0.763
5	201	0.0077	0.0071	1.5240	0.922
6	290	0.0042	0.0058	0.9932	1.381
7	247	0.0114	0.0133	1.2481	1.167
8	252	0.0056	0.0143	2.3474	2.554
\bar{X}	248	0.0087	0.0106	1.2543	1.316
S.E.	12	0.0013	0.0013	0.1774	0.194

accessible to the exchange between blood and brain and of the brain extravascular water content. The cerebral mean transit time was calculated according to the basic equation relating volume to flow ($\bar{t} = \text{CBV}/\text{CBF}$). The cardiac index (CI) was simultaneously and systematically determined according to the Stewart and Hamilton principle. Mean values of the different parameters for normal rats are collected in Table I. As far as CI and CBF are concerned, they are in the range of those listed in the literature [3, 4].

The relevance of our CBF and CBV indices to the cerebral circulation is particularly emphasized by the influence of various concentrations of CO_2 in inhaled air on their mean value. The effects of CO_2 on CBV and CBF both determined by using the non-invasive radiotracer subtractive method (Figs 1 and 2, solid lines) are respectively compared with the CO_2 effects on the cerebral blood volume determined in isolated brain (Fig.1, dashed line) and the effect of CO_2 on cerebral blood flow determined invasively (Fig.2, dashed line). These comparisons show that our procedure for CBV is more in agreement than that of the CBF determinations. However, our mean values presented in Fig.2 are in agreement with the conclusions of Kety and Schmidt [6] on the effect of CO_2 on CBF (CBF after 5% CO_2 is twice that found in animal breathing room air, and the maximum CBF increase is reached after 10% of CO_2).

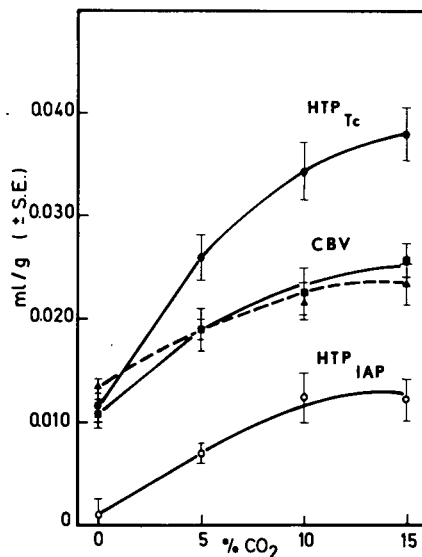


FIG.1. Effect of increasing concentration of CO_2 in the inspired air on the pool of Tc(HTP_{Tc}) and IAP(HTP_{IAP}) in the cephalic region. The difference measures the pool of Tc which leaves the cephalic region as it does not cross the blood brain barrier. This latter pool corresponds to the cerebral blood volume (CBV). The dashed line joins the experimental points obtained by direct measurement of the cerebral blood volume by the ^{51}Cr -labelled red blood cell method.

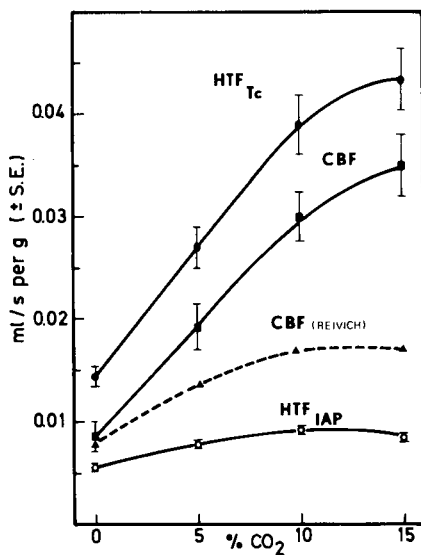


FIG.2. Effect of increasing concentration of CO_2 in the inspired air on the flow of Tc(HTF_{Tc}) and IAP(HTF_{IAP}) during their first passage through the head. The difference measures the cerebral blood flow (CBF). Dashed line drawn according to the experimental data of Reivich [5].

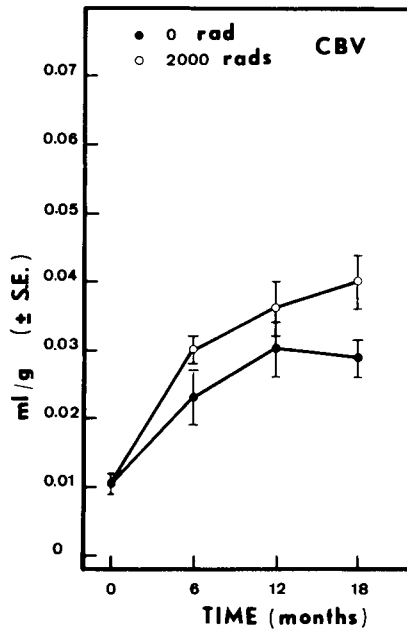


FIG.3. Variation in cerebral blood volume as a function of time after exposure in rats irradiated on the brain at 2000 rads, and in their controls.

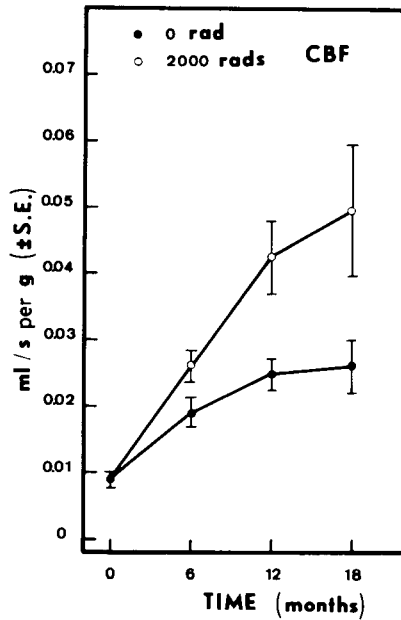


FIG.4. Variation in cerebral blood flow as a function of time after exposure in rats irradiated on the brain at 2000 rads, and in their controls.

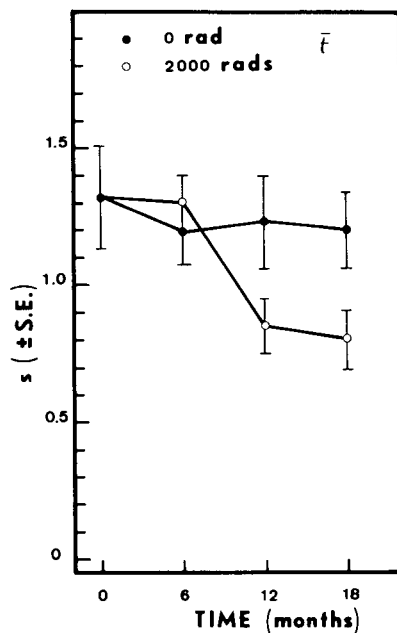


FIG.5. Variation in cerebral circulatory mean transit time as a function of time after exposure in rats irradiated on the brain at 2000 rads, and in their controls.

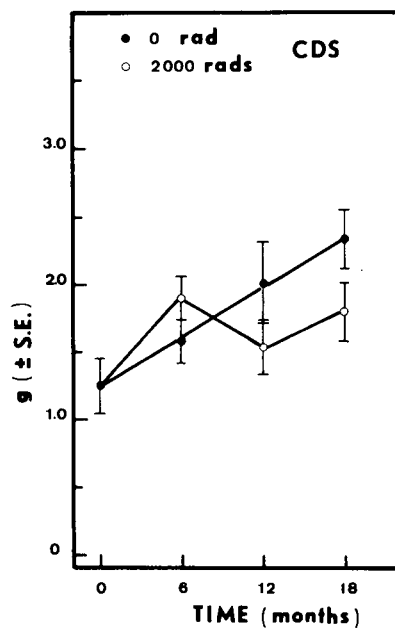


FIG.6. Variation in cerebral distribution space of iodoantipyrine as a function of time after exposure in rats irradiated on the brain at 2000 rads, and in their controls.

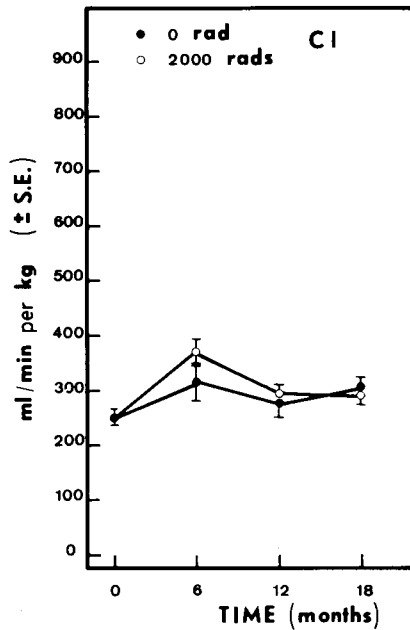


FIG. 7. Variation in cardiac index as a function of time after exposure in rats irradiated on the brain at 2000 rads, and in their controls.

RESULTS

The results of the measurements performed less than one month, 6, 12 and 18 months after irradiation are illustrated in Figs 3–7. The values of irradiated rats examined less than one month after exposure were found to be very close to those of the controls. For this reason they are quoted together at zero time in the graphs.

As shown in Fig. 3, the increase in the cerebral blood volume (CBV) characterizing the radiation effect is already evident at 6 months, and is particularly significant at 18 months after exposure. The general pattern of the late modification of the cerebral blood flow (CBF), as a function of time after exposure, emphasized the pattern observed for the late modification of the cerebral blood volume (Fig. 4). A very significant difference between control and irradiated rats is already reached 6 months after irradiation. The increase in CBV together with a comparatively higher increase in CBF is the reason why the circulatory mean transit time in the brain vessels (\bar{t}) shows a substantial decrease in its mean value from 6 months after irradiation. On the other hand, the effect of age on this parameter appears to be negligible (Fig. 5). As illustrated in Fig. 6, the cerebral distribution space of iodoantipyrine (CDS) is a linear function of age. It seems

that the action of radiation is in limiting the increase of this function at about 6 months after irradiation. From this time on, the brain content in iodoantipyrine is smaller in the irradiated than in the non-irradiated animals of the same age. At 18 months, although the depression of CDS is emphasized, the increment of CBF and CBV is lowered when it is compared with that observed at 12 months. The cardiac index (CI) of the control animals is not significantly modified by age before 21 months (Fig.7), and the cardiac index of the irradiated rats is very similar to that of the corresponding controls.

DISCUSSION

Several months after a relatively low dose of irradiation the cerebral circulation is paradoxically modified in a direction opposite to that of other systems which are generally depressed [7]. From six months after irradiation of the brain at 2000 rads, the cerebral blood flow (CBF) as well as the cerebral blood volume (CBV) are increased. The increase in CBV is in particularly good agreement with the increase in the brain vascular density which was found by Hopewell [8] in similarly irradiated rats. If we take into account that the cerebral circulation is highly regulated in order to protect this organ against a defective supply in oxygen as well as in essential nutrient substances, the observed modification in the circulatory dynamics could be interpreted as a response of the local circulatory system (the cardiac index is almost unchanged) to an accumulation in the parenchyma of products of catabolism and/or to a decrease in the amounts of oxygen and glucose which reach the grey and white matter cells. As shown by comparison between Fig.3 with Fig.1 and Fig.4 with Fig.2, the increases in CBV and CBF observed as a function of time after 2000 rads on the brain reproduce those caused by a progressive enhancement of CO₂ in inhaled air. In our opinion, the similar effect of CO₂ and radiation is indicative of an increasing hypoxia of the irradiated brain tissues. According to our results for CBV and CBF, this pathological process becomes evident between 6 and 12 months after exposure, although Reinhold [8], with the same experimental conditions, showed that gross vascular lesions appear after a latent period of 12 to 15 months. The existence of progressive structural and/or metabolical changes in the irradiated brain is also suggested by the modification of the cerebral distribution space of iodoantipyrine (CDS) which becomes smaller in irradiated rats compared with control animals. This reduction in CDS, which also begins between 6 and 12 months after exposure, could depict a decrease in the brain water content. This interpretation is supported by the parallel increase in hydroxyproline (used as an indicator of fibrosis) found by Gerber [8] in similarly irradiated brain. On the other hand, the decrease in CDS could also be the consequence of a change in the irradiated microvasculature restricting the diffusibility of iodoantipyrine, although this substance is freely diffusible through normal brain capillary

walls. In addition, our later observations suggest that the action of a compensatory mechanism which could explain the increase in CBF and CBV at 1 year after irradiation is at a maximum at 18 months, and no longer operating despite a more obvious lowering in CDS.

CONCLUSION

The late change in the cerebral circulatory dynamics after brain irradiation appears before any morphological modification of brain microvasculature. In this context, the cerebral blood volume and the cerebral blood flow increases could be interpreted as a reactive process against a progressive radio-induced modification disturbing the exchange between blood and brain. Where is the location of this disturbance: at the capillary level or in the complex metabolical pathways of the nervous cells? The answer to this question is a subject for the future.

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DISCUSSION

J.R. MAISIN: Your results show a change in the cerebral volume and in the blood flow in the brain after a dose of 2000 R to the head. Could this observation not be explained by the development of a collateral circulation in the brain?

A. KEYEUX: There is in fact an increase in the density of the capillaries, at least in the cerebral cortex (see Ref. [8] of the paper). In the absence of atrophy of the cortex this observation could be explained by the opening of capillaries, which up to then had not been functioning, accompanied by vasodilatation.

A.G. LURIE: Could radiation-induced vascular proliferation account for all or part of the increase obtained in vascular volume?

A. KEYEUX: Your question is very similar to that of M. Maisin. Radiation-induced vascular proliferation is an attractive but essentially speculative hypothesis, as no firm evidence is available. It is more reasonable to interpret the increase of the cerebral blood volume observed in our irradiated rats as the consequence of a compensatory mechanism developed by the brain circulation regulatory system.

H. FRISCHAUF: Have you considered the possibility that your results might have been affected by a radiation-induced increase in permeability? The observed prolongation of transit time and increase in CBF could be caused by augmented passage of labelled substances out of the cerebral vessels.

A. KEYEUX: That is a good question. However, as we did not observe any difference between the technetium contents at equilibrium of the cephalic regions of the irradiated and control animals, we assumed that the haematoencephalic flow pattern had not been affected by irradiation under our experimental conditions. The possibility that our measurements of cerebral blood flow may have been influenced by a radio-induced modification of the permeability of the cerebral capillaries can therefore be ruled out.

H.H. VOGEL: It might be of interest for your physiological studies of the rat brain to know that 50 rads of fission neutrons, given in a single dose to a female rat on her 18th day of gestation, causes tremendous damage to the central nervous system of the young born after irradiation. Much damage is seen to both cerebrum and cerebellum. The corpus callosum may be missing and the cortical layer is often reduced by 50% in thickness. Such morphological changes might make interesting correlations in your physiological measurements as evidenced by counting techniques. (I am personally studying behavioural changes in the young rats after birth.)

A. KEYEUX: What we have presented here is simply a pilot experiment using a non-conventional approach of CBV and CBF assessments. Additional experiments with measurements performed at different times after smaller and larger doses of X-rays (in order to obtain a relevant time-dose-effect relationship) are in progress.

Thank you very much for your interesting suggestion.

**THEORETICAL AND EXPERIMENTAL
STUDY OF THE DOSE-RESPONSE
RELATIONSHIP FOR LATE EFFECTS**

Session 8

FUNDAMENTAL ASPECTS OF CANCER INDUCTION IN RELATION TO THE EFFECTIVENESS OF SMALL DOSES OF RADIATION

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Abstract

FUNDAMENTAL ASPECTS OF CANCER INDUCTION IN RELATION TO THE EFFECTIVENESS OF SMALL DOSES OF RADIATION.

Analysis of dose-effect relationships for induction of malignant transformation of cells, of chromosome aberrations, and of cell reproductive death, and their dependence on radiation quality, suggests that the yield of tumours developing after irradiation can be described by

$$Y = (p_0 + p_1 D + p_2 D^2) \times e^{-a_1 D - a_2 D^2} \times F_{\text{immunol}}(D) \times F_{\text{hormon}}(D) \times F_{\text{tiss}}(D)$$

in which the last three factors represent nonstochastic effects which become significant at larger doses, e.g. in excess of a few hundred rads of X-rays. On the basis of a review of experimental data it can be concluded that for different types of cells a large variation is observed in the values of a_1 and a_2 which determine cell reproductive death. A review of data on chromosome aberrations suggests that the RBE of high-energy gamma radiation relative to 200–300 kV X-rays is approximately 0.5, i.e. significantly smaller than the value of 0.8–0.9 commonly observed for cell reproductive death. It is concluded that for tumour induction in different types of tissues, different values of the parameters in the formula for the yield must be applied in order to obtain an adequate description for the dependence on dose, dose rate, dose fractionation and radiation quality. Application of values derived from other radiobiological data shows that for experimental data on leukaemogenesis in mice, an adequate description can be derived. This implies that the model can also be considered as a useful basis for extrapolation to low dose rates and small doses for which no experimental data are available.

1. INTRODUCTION

Malignant transformation of cells by various agents and the subsequent proliferation kinetics which lead to the development of a tumour with unlimited growth capacity and invasive growth characteristics, involve a complex sequence of events that can be inhibited and promoted by a variety of factors and

conditions. Malignant transformation of mammalian cells may be caused by radiation, by viruses and by chemicals, and tumour development may be influenced by cell reproductive death, by immunological responses, by hormonal factors or by general tissue damage inducing proliferation of cells that have retained the clonogenic capacity. In addition, the type of tissue involved and the genetically determined susceptibility of the host may play an important part in the final yield of tumours from a given treatment. Insights into the mechanisms of tumour induction by ionizing radiations might contribute to a better understanding of carcinogenic processes in general, and studies of dose-effect relationships are therefore of even greater importance than might be deduced from consideration of radiation protection aspects only.

An important advantage of studies of radiation-induced biological effects, in contrast to effects initiated by viruses and chemicals, is that the magnitude and distribution of the dose can be determined and controlled with good accuracy, and that changes in effectiveness per unit dose can be studied as a function of dose rate, dose fractionation and radiation quality. These latter factors will be given special attention in the present report.

The development of various types of cancer as a consequence of irradiation of tissues in animals and man is a well-documented phenomenon for doses in excess of about 100 rads, and the influence of dose fractionation, dose rate and radiation quality has been studied in various experimental animals. Estimates of risks of cancer induction in man for such small doses and low-dose rates as are of major interest for radiation protection considerations must, however, be derived almost exclusively by extrapolation from data which are available for much larger doses and higher dose rates. This extrapolation should ideally be based on knowledge about mechanisms by which various types of damage are induced, and about the associated dose-effect relationships. Unfortunately this knowledge for malignant transformation of cells is not adequate to predict the shapes of dose-response curves, and dose-effect relationships must therefore be adopted on the basis of evidence about fundamental radiobiological processes deduced for other types of effects. If a model with parameters derived on this basis satisfactorily predicts the observed yield of tumours as a function of dose, dose rate, dose fractionation, radiation quality and other factors, it may be considered as an appropriate model to make predictions at levels of dose and dose rate for which data are not available.

2. GENERAL CHARACTERISTICS OF DOSE-EFFECT RELATIONSHIPS

For most radiobiological effects induced by X-rays or gamma radiation the frequency of a specified response, expressed as the fraction of cells affected in a large population, is not proportional to the dose but increases more rapidly

than a linear function. Taking a natural incidence p_0 into account, the yield of such an effect can be expressed as a first approximation by

$$Y = p_0 + p_1 D + p_2 D^2 \quad (1)$$

The linear and quadratic terms can be interpreted by assuming that the primary biophysical event involved can be induced either by energy deposition from a single ionizing particle or by energy deposited by more particles, passing through a given, for many effects as yet unspecified, site or structure in the cell.

Malignant transformation of cells can be considered as the consequence of inactivation or loss of heritable information required for the recognition of growth-regulating factors in a tissue or in an organism. Such a heritable change might be initiated in single cells according to a dose-effect relation such as formula (1), analogous to the relation observed, for instance, for some types of chromosome aberrations and mutations.

Since the expression of malignant transformation requires reproductive integrity of the transformed cell, the yield of tumours developing from these cells will be subject to modification, due to radiation-induced loss of the capacity for unlimited proliferation. This loss of reproductive integrity can be described by various formulas, but one of the most simple relations, which is frequently satisfactory for experimental data at doses up to a few hundred rads, corresponding to surviving fractions between 1.0 and 0.1, is given by

$$S_D/S_0 = e^{-a_1 D - a_2 D^2} \quad (2)$$

For other factors mentioned in the introduction, hormonal and immunological influences and severe tissue damage causing accelerated proliferation of surviving cells, adequate expressions are not known, and consequently the yield of tumours can be given only as

$$Y = (p_0 + p_1 D + p_2 D^2) \times e^{-a_1 D - a_2 D^2} \times F_{\text{immunol}}(D) \times F_{\text{hormon}}(D) \times F_{\text{tiss}}(D) \quad (3)$$

in which the last three factors are unknown functions of the dose of radiation. With respect to these latter factors, it can be argued that $F_{\text{immunol}}(D)$ and $F_{\text{hormon}}(D)$ as well as the function $F_{\text{tiss}}(D)$ represent nonstochastic effects which become significant only at doses in excess of a few hundred rads. It is likely that the initiation of these responses requires doses at which more than 50% of immunologically competent cells are killed or 50% of hormone production is impaired, and in addition these responses may require more than

a few days for their expression. Thus a threshold dose must be exceeded before a reaction will be induced which can either enhance or inhibit tumour development. Consequently it appears reasonable as a first approximation to ignore the influence of these factors for doses of up to a few hundred rads of X-rays, and for equivalent doses of other types of radiations.

With respect to the relative magnitudes of p_1 versus p_2 and a_1 versus a_2 in the formulas (1) and (2), it should be mentioned that linear terms become more dominant with increasing LET or \bar{Y}_F of the type of radiation considered. In this respect and with regard to the dependence on dose fractionation and dose rate, the available information on carcinogenesis demonstrates frequently qualitative similarities in comparison with other types of effects, e.g. chromosomal aberrations, cell reproductive death and tissue damage.

The function (3) for induction of a given type of cancer implies that the curve representing the incidence as a function of the dose tends to increase in slope with increasing dose at relatively small doses, but at larger doses a maximum will be attained with a subsequent decrease at doses where cell reproductive death becomes dominant.

3. DIFFERENCES IN DOSE-EFFECT RELATIONS FOR DIFFERENT EFFECTS IN MAMMALIAN CELLS

For two types of effects in mammalian cells a relatively large amount of data is available in the literature about the dose-effect relationships and the dependence on radiation quality, namely cell reproductive death and the induction of chromosome aberrations. Similarities and differences between relationships for these two types of effects have been discussed elsewhere to demonstrate relations between various parameters that can be obtained from the experimental data [1–5]. A few conclusions will be summarized.

3.1. Cell reproductive death

It is now generally recognized that survival curves obtained for different types of mammalian cells irradiated with X-rays can vary considerably with respect to their shape and slope. Bone marrow stem cells are more sensitive than mouse intestinal crypt stem cells, whereas cells from a mouse lymphocytic leukaemia L5178Y have been shown to be more sensitive than cells from a rat rhabdomyosarcoma [2]. The differences observed pertain to the shape and slope in the low-dose region as well as to the slope at larger doses corresponding to fractions of surviving cells of less than 0.1, where the curves frequently are indistinguishable from exponential [3]. As noted earlier, the shapes of many survival curves in the low-dose region can be described satisfactorily by

formula (2). An analysis of many survival curves for X-rays and gamma rays shows that values of a_1 range from about 10^{-3} to 10^{-2} rads $^{-1}$, whereas values of a_2 range from 10^{-6} to 10^{-5} rads $^{-2}$ [5]. In view of these large variations it is no longer justifiable to suggest that mammalian cells all have similar sensitivities for cell reproductive death. Furthermore, calculations of mean values of a_1 and a_2 have little significance. Values of a_1/a_2 also show a wide range from 300 to 1200 rads. Values of a_1 and a_2 for high-energy gamma rays are generally only a factor of 0.8 to 0.9 smaller than those for 200–300 kV X-rays.

For high LET radiation, a comparison of published data for different cell lines shows also large differences in a_1 , ranging from 0.8×10^{-2} rads $^{-1}$ to 3×10^{-2} rads $^{-1}$ for LET values corresponding to maximum effectiveness [5]. It can be concluded that with respect to the contribution of cell reproductive death to modification of yield versus dose relationships for tumour induction for each type of tissue considered, the relevant cell survival curve parameters must be taken into account.

3.2. Chromosome aberrations

For chromosome aberrations data are available for a smaller number of types of cells than for cell reproductive death, but these are sufficient to show significant differences between corresponding parameters in comparison with cell reproductive death.

Data on the induction of chromosome structural changes observable at mitosis, e.g. dicentrics, centric rings and acentric fragments, indicate that values of p_1 for high LET radiation in human lymphocytes are not much smaller than values of a_1 for cell reproductive death in the same type of cells. This indicates that at high LET or \bar{Y}_F , cell reproductive death may be associated with a frequency in excess of 0.5, with severe chromosome structural changes. However, for low LET radiations the values of p_1 for the same type of damage are much smaller than the corresponding values of a_1 [5]. In addition, values of p_1 are significantly smaller by a factor of about 2 for high-energy gamma rays compared with 200–300 kV X-rays. These differences are associated with larger maximum RBE values of high LET radiation observed for the linear component of chromosome aberrations induction, ranging up to values of about 40, compared with maximum RBE for cell reproductive death ranging up to about 10 [5].

With respect to the values of a_2 and p_2 for low LET radiations, derived for a variety of types of cells, differences are smaller than between corresponding values of a_1 and p_1 . Furthermore, differences between 200–300 kV X-rays and high-energy gamma rays with respect to a_2 and p_2 appear to be of the same magnitude as for a_1 . As a consequence, values of p_1/p_2 for low LET radiations are frequently a factor of 5–10 smaller than values of a_1/a_2 . Moreover, significant differences by a factor of 2 can be deduced between p_1/p_2 values for high-energy

TABLE I. COMPARISON OF PARAMETERS DERIVED FROM EXPERIMENTAL DATA FOR DIFFERENT TYPES OF MAMMALIAN CELLS

<i>Cell reproductive death</i>	<i>Chromosome aberrations (dicentrics, centric rings, fragments)</i>
$S_D/S_0 = e^{-a_1 D - a_2 D^2}$	$Y = p_1 D + p_2 D^2$
<i>X-rays (200–300 kV)</i>	<i>X-rays (200–300 kV)</i>
a_1 range: $10^{-3} - 10^{-2}$ rads ⁻¹	p_1 range: $10^{-4} - 10^{-3}$ rads ⁻¹
a_2 range: $10^{-6} - 10^{-5}$ rads ⁻²	p_2 range: $10^{-6} - 10^{-5}$ rads ⁻²
a_1/a_2 : 300–1200 rads	p_1/p_2 : 70–300 rads
<i>Gamma rays (~ 1 MeV)</i>	<i>Gamma rays (~ 1 MeV)</i>
RBE ~ 0.8–0.9 for a_1	RBE ~ 0.3–0.6 for p_1
RBE ~ 0.8–0.9 for a_2	RBE ~ 0.8–0.9 for p_2
<i>High LET radiations</i>	<i>High LET radiations</i>
max. a_1 range $(0.5-3) \times 10^{-2}$ rads ⁻¹	max. p_1 range $(0.2-2) \times 10^{-2}$ rads ⁻¹
max. RBE ~ 10	max. RBE ~ 20, relative to X-rays
	max. RBE ~ 40, relative to gamma rays

gamma rays compared with 200–300 kV X-rays. For dicentrics induced in human lymphocytes these values are about 50 rads and 100 rads respectively. A summary of various characteristic parameters is given in Table I.

4. INTERPRETATION OF YIELD VERSUS DOSE CURVES FOR TUMOUR INDUCTION

The conclusion that significant differences are observed among various types of cells with respect to the values of parameters which characterize the dose-response relations for reproductive death and that in addition values of parameters for chromosome aberrations differ from corresponding values for reproductive death, implies that for the interpretation of yield versus dose curves for tumour induction unambiguous indications about the values of parameters in formula (3) cannot be obtained. Nevertheless, for some specific cases it is of interest to apply the available data in order to analyse whether, with formula (3) and on the basis of the parameters selected, changes in effectiveness due to

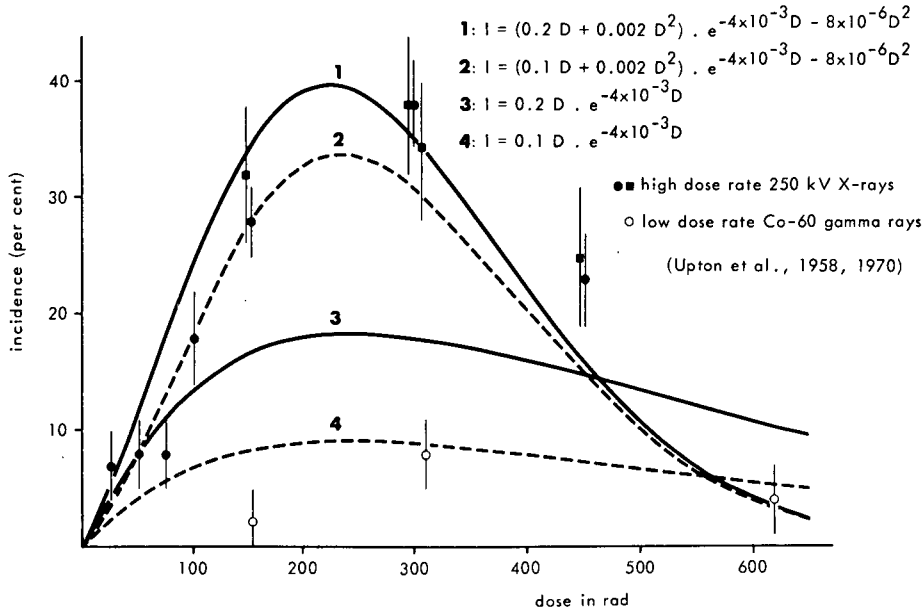


FIG.1. Comparison of experimental data on induction of myeloid leukaemia in ♂ RF mice, from Refs [6, 7], with theoretical curves according to the formulas indicated.

Curves 1 and 2: high dose rate, 200–300 kV X-rays and ^{60}Co gamma rays, respectively.

Curves 3 and 4: low dose rate, 200–300 kV X-rays and ^{60}Co gamma rays, respectively.

variations in dose rate, dose fractionation and radiation quality can be interpreted. As an example, such an analysis will be applied to leukaemogenesis in experimental animals. A suitable set of data on induction of leukaemia in mice with various irradiation schemes and different radiation qualities has been published by Upton and co-workers [6, 7]. For this myeloid leukaemia the radiosensitivity with respect to reproductive death of the susceptible normal cell type can be assumed to be equal to the sensitivity of bone marrow stem cells, for which data are available. In order to analyse whether formula (3) can adequately represent the yield of these leukaemias as a function of dose, dose rate, dose fractionation and radiation quality, appropriate values of the parameters a_1 , a_2 , p_1 and p_2 will be adopted on the basis of experimental data available from other sources [5]. For mouse bone marrow stem cells values of a_1 and a_2 equal to $4 \times 10^{-3} \text{ rads}^{-1}$ and $8 \times 10^{-6} \text{ rads}^{-2}$ respectively will be applied for 200–300 kV X-rays as well as for gamma rays. Assuming an analogy with chromosome aberration induction, rather than with reproductive death, for induction of malignant transformation, values of p_1/p_2 equal to 100 rads for 200–300 kV X-rays and of 50 rads for ^{60}Co gamma rays will be adopted.

In Fig. 1, the observed yields of myeloid leukaemias in male RF mice irradiated with single doses of X-rays and with low-dose rate gamma rays are presented [7]. In this figure, curve 1 represents the calculated yield versus dose curve obtained with the values of a_1 , a_2 and p_1/p_2 adopted, and with a value of $p_1 = 0.2 \text{ rads}^{-1}$ for X-rays derived by an approximate fitting procedure for the experimental data for single doses. If for 200–300 kV X-rays and high-energy gamma rays values of p_2 are assumed to be approximately equal, it follows that p_1 is 0.1 rads^{-1} for gamma rays, i.e. half of the value for X-rays. This value was employed for the calculation of curve 4 for low-dose rate gamma radiation. The results show a very flat response for protracted exposures, in fair agreement with the observed yields.

It is evident that the general shape of curve 1 is in reasonable agreement with the incidences observed after single doses of X-rays. This is important because except for the parameter p_1 , the values employed in formula (3) have been derived from independent data on cell reproductive death and from an assumed analogy between the malignant transformation and induction of chromosome aberrations with respect to the ratio p_1/p_2 . An almost perfect fit to the observed incidence can be obtained by assuming a value $p_1/p_2 = 50 \text{ rads}$ and values of $a_1 = 3.6 \times 10^{-3} \text{ rads}^{-1}$ and $a_2 = 7.2 \times 10^{-6} \text{ rads}^{-2}$, but this is not shown in Fig. 1 because the purpose of this analysis is not to show simply that a good fit can be obtained by optimal selection of the four parameters, but to demonstrate that the application of parameters derived on the basis of other types of experimental evidence provides a good approximation of the data on leukaemia induction with the model adopted.

In order to illustrate the importance of the assumption that values of p_1/p_2 are a factor of 2 smaller for high-energy gamma radiation than for 200–300 kV X-rays, curves 2 and 3 for high-dose rate gamma rays and low-dose rate X-rays have been included in Fig. 1, calculated with the same values of the parameters p_2 , a_1 and a_2 employed to derive curves 1 and 4 respectively. This shows that a significantly larger effectiveness for low-dose rate X-rays compared with gamma rays would have been expected, whereas for high-dose rate gamma irradiation the difference for 200–300 kV X-rays is much smaller and would have been difficult to demonstrate.

In addition to experimental data on leukaemia induction by single acute doses of X-rays and by low-dose rate irradiation with gamma rays, results have been published on the effectiveness of fractionated treatments [6]. Using the same parameters as adopted for the interpretation of the effectiveness of single doses, calculations can be made to predict the effect of fractionated doses. The results are presented in Table II, and show a fair agreement with the observed incidence. It is evident that the incidence observed after three fractions of 150 rads is not significantly different from the incidence after a single dose of 150 rads or the incidence after a single dose of 450 rads, and this at first sight

TABLE II. EFFECT OF FRACTIONATION ON LEUKAEMIA INCIDENCE IN RF MICE ♂ INDUCED BY X-RAYS

Model: $I = (0.2 d + 2 \times 10^{-3} D^2) \times e^{-4 \times 10^{-3} D - 8 \times 10^{-6} D^2}$		
	Incidence (%)	
	Calculated	Observed
75 rads, single dose	19	8 ± 3
150 rads, single dose	34	32 ± 6
150 rads, 2 fractions, 2 days interval	26	15 ± 5
150 rads, 2 fractions, 6 days interval	26	22 ± 5
450 rads, single dose	16	25 ± 6
450 rads, 3 fractions, 2 days interval	22	20 ± 6
450 rads, 3 fractions, 5 days interval	32	26 ± 7

Calculation	Induced leukaemia (%)	Cell reproductive integrity
Effect of 1st dose of 75 rads	18.6	0.71
Effect of 2nd dose of 75 rads	$18.6 \times 0.71 + 0.71 \times 18.6 =$ 26.4	0.50
Effect of 1st dose of 150 rads	34.4	0.46
Effect of 2nd dose of 150 rads	$34.4 \times 0.46 + 0.46 \times 34.4 =$ 31.7	0.21
Effect of 3rd dose of 150 rads	$31.7 \times 0.46 + 0.21 \times 34.4 =$ 21.8	0.10
Effect of 3rd dose of 150 rads, assuming repopulation to 0.5	$31.7 \times 0.46 + 0.5 \times 34.4 =$ 31.8	0.25

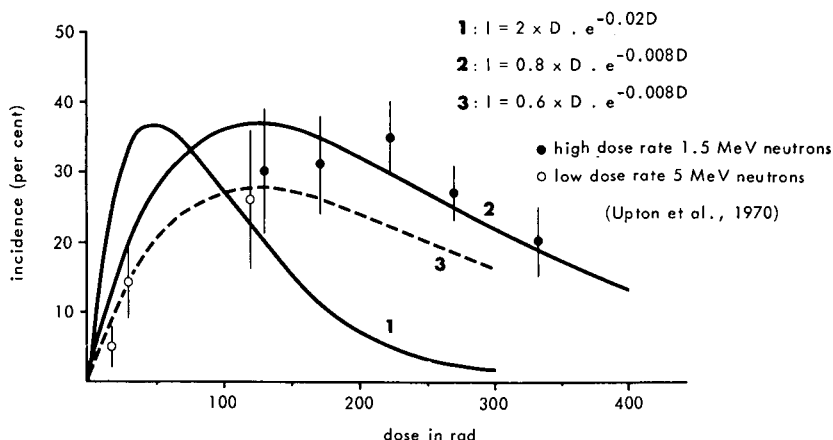


FIG.2. Comparison of experimental data on induction of myeloid leukaemia in ♂ RF mice, from Ref. [7], with theoretical curves according to the formulas indicated.

Curves 1 and 2: high dose rate, 1.5 MeV neutrons. Curve 3: low dose rate, 5 MeV neutrons.

surprising result of fractionation is in good agreement with predictions on the basis of formula (3), using the same values of parameters adopted for the description of the curve 1 of Fig.1 for single doses of X-rays. For the long interval of 5 days, some repopulation of the surviving bone marrow stem cells to 0.5 of the initial number has been assumed on the basis of data by Lahiri and co-workers [8].

In addition to experimental data on leukaemia induction by X-rays and gamma rays, results have been published on the effectiveness of fast neutrons applied at high- and low-dose rates. Unfortunately for the high-dose rate experiments, neutrons with a mean energy of 1.5 MeV were employed whereas for low-dose rate experiments neutrons with a mean energy of about 5 MeV were applied. Because of differences in RBE to be expected as a function of neutron energy, a direct comparison of these results may not be justified [7].

For the neutrons applied at a high-dose rate, no corresponding data with respect to the effectiveness for reproductive death of mouse bone marrow stem cells have been published. Furthermore, uncertainties concerning dosimetric parameters, the neutron energy spectrum and the contribution of gamma rays must be taken into account for an estimation of RBE values [9]. As a consequence it is difficult to predict values of p_1 and a_1 , but as an approximation an RBE value of p_1 equal to 4–10 relative to 200–300 kV X-rays, and for a_1 an RBE value of 2–5 will be considered. Values p_2 and a_2 will be assumed to be sufficiently low to play an insignificant part at the doses employed. On the basis of this selection of ranges of RBE values for p_1 and a_1 , the curves 1 and 2 have

been calculated for the two extreme values. The results of the calculations of incidence versus dose are shown in Fig.2. It is evident that curve 1 is not compatible with the observed incidences, but curve 2 provides a good approximation. For the higher energy neutrons applied at a low dose rate, an RBE value for p_1 equal to 3 relative to X-rays has been employed in order to provide a better fit than obtained with an RBE value of 4. This lower RBE is in agreement with the general trend of the dependence of RBE on neutron energy.

From a comparison of the curves for fast neutrons in Fig.2 with the curves of X-rays and gamma rays in Fig.1 it can be derived that at low doses and dose rates the RBE of 1.5 MeV neutrons would be equal to 4 relative to 200–300 kV X-rays and 8 relative to high-energy gamma radiation. For 5 MeV neutrons these values would be 3 and 6 respectively.

It can be concluded that the model described by formula (3), with parameters adopted on the basis of analogy with other radiobiological data, can adequately describe variations in effectiveness for leukaemogenesis in mice in dependence on dose, dose rate, dose fractionation and radiation quality. This implies that this model can be employed also to derive the effectiveness at small doses and very low-dose rates, where no experimental data are available. A similar model, using appropriate parameters, can be adopted to analyse the effectiveness of small doses for tumour induction in man on the basis of evidence obtained at larger single doses.

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DISCUSSION

P.G. GROER: In your formula for Y there is no time-dependence indicated. At what time should Y be evaluated?

G.W. BARENDSEN: In the concepts from which the formulas were derived, it is assumed that the total incidence, i.e. integrated over the lifetime of the animals, corrected for intercurrent death from other causes, can be described as a function of dose, dose rate, dose fractionation and LET. This will most likely not be correct for experimental data where the incidence increases to close to 100%, or where intercurrent death kills more than 50% of the animals.

H.H. VOGEL: If I read your figures correctly, I believe that you show a maximum value for linear energy transfer (LET) of 10^2 or above ($\text{keV}/\mu\text{m}$) for chromosomal aberration data in mammalian cells. What radiations were used besides the X- and gamma- low LET radiations and the fast neutrons you mentioned in describing the beams? Did you include any high-energy ions in this work?

G.W. BARENDSEN: The data which showed a distinct peak in effectiveness for chromosome aberrations between 100 and 400 $\text{keV}/\mu\text{m}$ were obtained by Skarsgard and co-workers for cultured cells irradiated with heavy ions at about 10 MeV energy per nucleon from the heavy ion accelerator at Yale University, New Haven, Connecticut. LET_∞ was varied by using different ions up to neon.

C. STREFFER: It is fascinating how your calculated data on induction of leukaemia agree with the observed cases. There are some other biological factors which we know of and I wonder whether you could include them and thereby improve the calculations. You are certainly familiar with the data from studies on cell transformation in vitro. Apparently DNA has to be taken up into transformed cells, which have to proliferate within a few days and not only survive and proliferate later in order to manifest the transformation. This concept is also in agreement with the induction of mammary tumours in animals and humans where proliferating breast tissue is more sensitive than non-proliferating tissue.

G.W. BARENDSEN: The studies on transformation in vitro yield transformation frequencies which are much larger than can be deduced, e.g. from the probability of induction of leukaemia. Thus only a very small fraction of the transformed cells will actually give rise to a tumour, and this small fraction may be made up of those that do retain the genetic change involved for a longer period than a few days. Later proliferation is of course required to yield a tumour. There is no discrepancy with data on mammary tumour induction because, if proliferation is accelerated, a higher incidence is expected if the genetically altered cells lose the changed characteristics slowly with time.

H. WIJCKER: The chosen equations for Y and S/S_0 describe the experimental results fairly well by a good choice of the parameters. Have you tried deriving these equations from a number of differential equations in which dy/dD and dS/dD are written as functions of D , Y and S , similarly to the way described by Sacher and co-workers¹ in this session?

Such equations would give more insight into the underlying mechanism than the integrated form presented here.

G.W. BARENDSEN: I do not think it is possible to derive these equations in a meaningful way as the solution of a number of differential equations. For chromosome aberrations that increase more rapidly than proportionally to the dose, I simply assumed a contribution from a component that increases with the square of the dose.

¹ SACHER, G.A., TYLER, S.A., TRUCCO, E., "The quadratic low-LET dose-effect relation for life shortening in mammals: Implications for the assessment of the low-dose hazard to human populations", these Proceedings 2, IAEA-SM-224/408.

SPONTANEOUS MUTATION RATES AND THE RATE-DOUBLING DOSE

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Abstract

SPONTANEOUS MUTATION RATES AND THE RATE-DOUBLING DOSE.

The amount of radiation required to double the frequency of mutations or tumours over the rate of those that occur spontaneously is called the rate-doubling dose. An equivalent concept has been proposed for exposures to other environmental mutagens. The doubling dose concept is predicated on the assumption that all human populations have the same spontaneous mutation rate, and that this spontaneous mutation rate is known. It is now established for prokaryotes and lower eukaryotes that numerous genes control the spontaneous mutation rate, and it is likely that the same is true for human cells as well. Given that the accepted mode of evolution of human populations is from small, isolated groups of individuals, it seems likely that each population would have a different spontaneous mutation rate. Given that a minimum of twenty genes control or affect the spontaneous mutation rate, and that each of these in turn is susceptible to spontaneously arising or environmentally induced mutations, it seems likely that every individual within a population (except for siblings from identical multiple births) will have a unique spontaneous mutation rate. If each individual in a population does have a different spontaneous mutation rate, the doubling dose concept, in rigorous terms, is fallacious. Therefore, as with other concepts of risk evaluation, the doubling dose concept is subject to criticism. Nevertheless, until we know individual spontaneous mutation rates with precision, and can evaluate risks based on this information, the doubling dose concept has a heuristic value and is needed for practical assessment of risks for defined populations.

INTRODUCTION

Mutations arise spontaneously at a given rate. The amount of radiation or chemical mutagen required to double the frequency of mutations in a population in one generation is called the rate-doubling dose. The doubling dose is used as a crude guide to the amount of genetic damage that society could sustain without it being an intolerable burden. For man, the spontaneous mutations are responsible for an incidence of approximately eight to ten per cent of known genetic defects in each generation [1, 2]. These include dominant gene mutations, chromosomal abnormalities, recessive gene mutations, sex-linked mutations and multifunctional abnormalities. Of course, most of these are not severe. When an individual or group of individuals has been exposed to a mutagenic agent, at the doubling dose or higher, then procedures should be introduced to lessen the burden to society. The doubling dose has been accepted as one of the best standards for setting limits to exposures of radiation [3], and also has been suggested by Committee 17 [4] to be reasonable for establishing limits to exposures to environmental chemical mutagens. Advisory bodies use the estimated doubling dose to suggest conservatively limits of exposure at some small fraction of the doubling dose.

The best preventative that society has at present for keeping the frequency of genetically defective individuals within tolerable limits is by amniocentesis followed by induced abortion when defective embryos are detected. Amniocentesis is expensive. The standard for strongly recommending amniocentesis, and, if necessary, induced abortion, is based on the probability of increased frequencies of birth defects, whether the expected increase comes from exposure to a mutagen where amniocentesis can be used, or to a teratogen where amniocentetic methods have not yet been developed. A version of the rate-doubling dose concept has already become a reality for age, a 'natural mutagen', where the incidence of children born with Down's syndrome begins to rise as a function of the age of the mother. Amniocentesis often is recommended by physicians for women who become pregnant in their middle thirties, at an age where the frequency of Down's syndrome among offspring has approximately doubled over that from pregnancies among women who are in their middle twenties (Fig.1).

THRESHOLDS

By now everyone is aware that the malignant transformation of cells and the induction of mutations are stochastic processes, whereas teratogenesis and poisoning, for example, are largely nonstochastic. Consequently, it is often stated that there are no threshold doses for the induction of mutations, whereas non-stochastic processes may exhibit true physiological, developmental or biochemical thresholds. Nevertheless, a detectability threshold does exist for mutations.

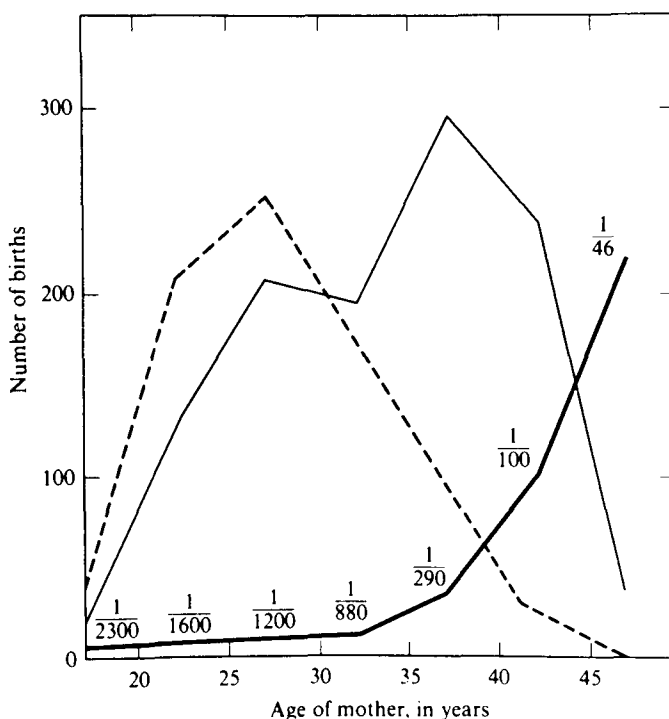


FIG.1. Incidence of Down's syndrome as a function of the mother's age. Dashed line: all births in thousands. Thin solid line: number of babies born with Down's syndrome. Thick solid line: incidence of Down's syndrome relative to maternal age ([5] after [6]).

The spontaneous mutation rate is a background 'noise' level from which the induced non-threshold effect will start. That is, the spontaneous mutation rate establishes a detectability threshold. An example of the spontaneous mutation rate as a detectability threshold is shown in Fig.2. It can be seen that the spontaneous rate for recessive lethal mutations on the X chromosome of *Drosophila* amounts to about 0.1% per generation. The average background radiation level is so low that it cannot induce more than one in ten thousand of these spontaneous mutations if we assume one target kinetics for mutation induction at doses below 25 rads. When the spontaneous mutations are subtracted from the induced, then the one target kinetics is maintained below the spontaneous level. This justifies an extrapolation. Not until the radiation dose to the *Drosophila* is raised to over one-tenth of the spontaneous rate could an enhancement in the mutation frequency be observed, and then it still remains a practical problem because a very large population of treated *Drosophila* would have to be analysed in order to obtain statistically significant results. A subtle point arises in the consideration of this problem. It is the difference between 'practical' and 'detectability' thresholds.

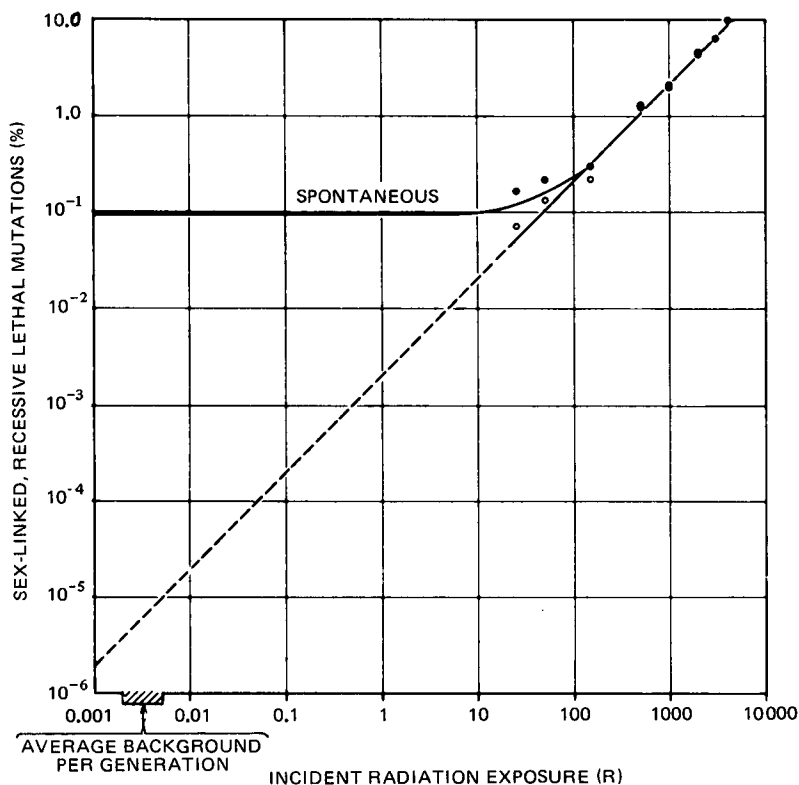


FIG. 2. Sex-linked, recessive lethal mutation frequency of *Drosophila* plotted as a function of incident radiation exposure. The dose-action curve has been extrapolated to indicate the proportion of spontaneous mutations that might be induced by background radiation (from [7]).

TABLE I. COSMIC RADIATION AS A FUNCTION OF ALTITUDE
(average value in millirads per year [9])

Altitude (metres)	Latitude 50°	Latitude near 0° (near equator)
0	41	35
1500	66	44
3050	128	89
4580	263	175
6100	500	340

TABLE II. ANNUAL DOSES TO GONADS IN DIFFERENT REGIONS WITH DIFFERENT BACKGROUND RADIOACTIVITY [9]

Region	Millirems per year
Normal (alluvial plains)	75 (45–90)
Granitic ground and houses (France)	190 (180–350)
Monazite region (Brazil)	315
Monazite region (Kerala coast, India)	830 ^a

^a A more recent estimate is 500 millirems per year for individuals living in this region [10].

A practical threshold is the amount of the mutagen under consideration given at a level below which no significant increase of the mutation rate over the spontaneous one is observed [1, 8]. The detectability threshold is a 'noise' level below which an effect cannot be observed readily. For example, using *Drosophila* again, analysis of about 7000 flies are required to obtain enough data for the statistical significance necessary to demonstrate a doubling dose of a mutagen. To recognize as statistically significant two induced mutations among ten thousand spontaneous mutations as opposed to one induced mutation in the control population would require scoring the flies in two populations each consisting of 8×10^{11} individuals (T. Kirkwood, personal communication).

The value of considering a practical threshold or a detectability threshold for the genetic effects of radiation or other mutagens can be seen by considering the data shown in Tables I and II. The amount of cosmic radiation is shown as a function of altitude in Table I. The amount of radiation as measured in millirads per year is increased by about 2.5 between sea level and 3000 metres near the equator, and just about triples at a latitude of 50°. In 1959 three cities at high altitudes were recommended for study for the incidence of genetically inherited disorders. These were Bogota at 2600 metres, La Paz at 3600 metres and Quito at 2850 metres. The studies have yet to be undertaken.

Table II shows data for annual doses to gonads of people who live in regions of the earth where different amounts of radiation are found. These data are corrected; it is assumed that transmission through the tissues to the gonads has an efficiency of 0.63, and that cosmic radiation accounts for 28 millirems per year at each location.

In France, granite increases the dose to the gonads by a factor of 2.6, and the ratio between dwellings of stone and wood in Norway is 3, in Sweden is

3.5, and in Boston is 5. Only in the Kerala region of India has the enhancement of birth defects (Down's syndrome) been suggested as being statistically significant [11], and even this suggestion is disputed [12, 13]. Individuals in the Kerala region are exposed to approximately 15 rads per generative lifetime of 30 years, not counting secondary exposures from absorption of radioactive compounds from foodstuffs grown there. The doubling dose for man is considered to be about 100 rads [3, 13]. This means that the radiation equivalence for spontaneous disorders is 100. The detectability threshold where an excess of defects is essentially not observable in humans is about ten per cent of this amount, or ten rads given over a 30-year period. The individuals on the Kerala Coast receive a dose just above the detectability threshold.

SPONTANEOUS MUTATION RATE IN MAN

Cavalli-Sforza and Bodmer [5], DeMars [14], and Morrow [15] have pointed out some of the obstacles that lie in the way of estimating reliable spontaneous mutation rates for man. These include errors introduced from lack of fitness of heterozygotes, from mutations being somatic rather than gametic, from phenocopies, from multiple loci determining similar conditions, from incomplete penetrance and from calculations of numbers of gonial cell divisions. Some of these errors may be negligible but some can be large. Nevertheless, Cavalli-Sforza and Bodmer [5] undertook the task of eliminating these errors in their estimate of the mean spontaneous mutation rate of 3.1×10^{-7} mutations per locus per generation. Morrow [15] estimates the mean spontaneous mutation rate for human cells in culture to be 7×10^{-7} mutations per locus per cell division. A number of loci were used to make these estimates, but there were no overlapping loci available so that estimates for cell populations and human populations could be compared directly.

What is needed are mutation rate studies in man and human cell cultures where rates are measured for the same genetic loci. It may be that the meiotic process itself can enhance the spontaneous mutation rate as it does in yeast and some other species [16–18]. Also the spectrum of types of spontaneous mutations may vary in gametic stages, i.e. frameshift mutations are predominant in yeast during meiosis, and base substitutions are predominant during mitosis.

The spontaneous mutation rate in man probably derives from the same sources as the spontaneous mutation rate in other eukaryotes and prokaryotes, that is, through replication, repair and recombination of DNA [7, 17, 19]. In addition the 30-year generative lifetime of man places him in a position to receive more environmental radiation, which he cannot avoid, and environmental chemical mutagens, which he could avoid, than, for instance, a *Drosophila* with a two-week generative lifetime or a yeast cell with a ninety-minute generative lifetime.

It has been stated that roughly 80% of human neoplasms depend directly or indirectly on environmental factors [20]. It is possible that any substance or radiation which causes an enhancement of a variety of neoplasms also will induce heritable disorders. Substances which give rise to one type of neoplasm or are not deeply penetrating (such as ultraviolet radiation) do not constitute a genetic hazard. Let us consider one source of cancer, cigarette smoke. Eight per cent of the deaths in the United Kingdom are caused by lung cancer [20], and lung cancer was a rare disease before soldiers were given free cigarettes during World War I. But cigarette smoke also seems to be involved in the aetiology of other cancers. Whether the same substances in the cigarette smoke that cause cancer also induce heritable mutations is unknown, but certainly mutagenic substances can be found in the urine after a cigarette has been smoked [21], indicating that these substances have retained their potency while circulating through the body fluids.

Consequently, a case can be made that a significant proportion of the 'natural' mutation rate in man may derive from factors in the environment. Nevertheless, some mutations certainly originate from intracellular mechanisms, most of which in eukaryotes seem to stem from mutagenic repair of lesions which take place during the replication of DNA [22].

NATURAL VARIATION OF THE SPONTANEOUS MUTATION RATE

Although the more obvious genetic defects induced in human populations by radiation and chemical mutagens are a burden to society, it is possible that a greater burden would ensue from induction of mutations which would increase the spontaneous mutation rate. These would enhance the genetic burden every generation henceforth. Mutations which cause rate alterations are most often caused by defects in genes which encode enzymes involved in repair of DNA.

The genetic diseases involving defects in DNA-repair systems are turning out to be common and widespread in human populations. Xeroderma pigmentosum and ataxia telangiectasia are the best known clinically of the DNA-repair diseases, but others are known or suspected [23]. Mutants are known in the yeast *Saccharomyces cerevisiae* for at least 50 different genetic loci which encode enzymes engaged in the repair of DNA. Not all of the loci encoding repair enzymes in yeast have been found; there may be as many as 100 genes involved. About 20% of the repair-defective mutants affect the spontaneous mutation rate, and most of these cause an enhancement of the rate rather than a lowering of it [24]. It is likely that at least as many repair-defective loci will be found in humans or human cell lines as there are in yeast. Undoubtedly many of these also will affect the spontaneous mutation rate or the sensitivity to different kinds of mutagens [25].

It is known that different strains of mice exhibit different incidences of spontaneous or induced tumours. It is to be expected that different strains of

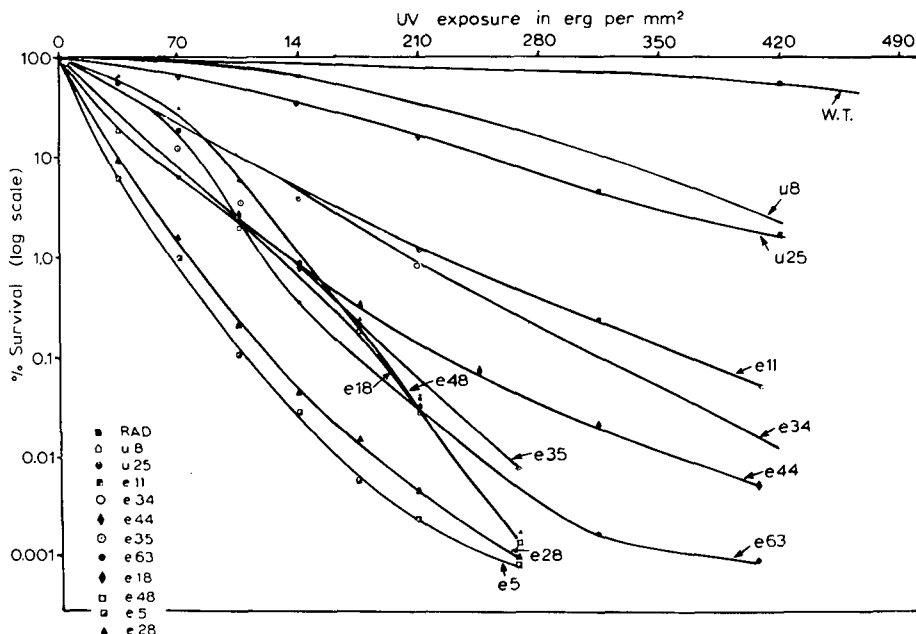


FIG. 3. Comparison of survival to ultraviolet radiation of a radiation-insensitive strain and strains comprising eleven different radiation-sensitive mutant alleles of the *rad 3* locus (from [27]).

mice should exhibit different spontaneous mutation rates and altered sensitivity to mutagens. Some of the sensitivity differences could be attributed to deficiencies in repair of DNA and others to alteration of enzymes that activate premutagens.

The enzymes that activate precarcinogens into carcinogens (or premutagens into mutagens), and those which control absorption, metabolism and excretion of premutagens, present great possibilities for genetic variation among individuals. A possible example of this was seen in a study by Yakovenko and co-workers [26]. Chromosome aberration frequencies were determined from peripheral leucocytes of cancer patients before and after therapy with thiophosphamide. A five-fold variation in individual response to thiophosphamide was found in the 59 cases studied. It is not known if this means that thiophosphamide in higher quantities should be given to the least responsive individuals, or if the secondary response (chromosome breakage) to the compound is simply five times less than the primary therapeutic action in the least sensitive individuals.

Mutants of genes that determine mutagen sensitivity can behave subtly. A replaced amino acid in a polypeptide chain may have little overall effect on enzymatic activity (that is, it would be a 'leaky mutant'), but it may endow the organism with a slightly different, yet measurable, sensitivity to a mutagen, or a slightly different spontaneous mutation or tumour rate. Although differences

can be very slight they may be measurable because the inherent accuracy in measurement of dose-response curves and spontaneous mutation rates is much greater than the accuracy of measurement of a physiological variable such as those caused by diet or by a disease. The array of sensitivities shown for different mutant alleles of different leakiness in the *rad3* locus of yeast is shown in Fig.3. It is presumed that the least sensitive mutant reflects a single amino acid change in protein, and the most sensitive mutant is a large deletion, a frameshift mutation or a nonsense mutation. We also know that different alleles of the same mutator locus can show different levels of enhancement of the spontaneous mutation rate [18], another indication of leaky alleles.

Another genetic phenomenon is known which can contribute substantially to variation of the spontaneous mutation rate or to sensitivity of a cell to radiation. A modifier gene which itself shows little effect can contribute a sizeable action in combination with, for example, a radiation-sensitive mutant. These can be the source of the variability contributed by the so-called genetic 'background' found in various genetic stocks. The actions of modifier mutants are easily recognizable even though they are troublesome to sort out by conventional Mendelian genetic methods. Modifiers can function at many levels, e.g. pH at a local site in a cell may be altered, osmotic pressures may be slightly shifted, temperature responses of an otherwise normal enzyme might be changed, or there may be metabolic changes of a specific or general nature. Modifier effects are found commonly when one works with the strain sensitivity to ultraviolet radiation in yeast (Fig.4), and these serve to confound experiments even with this genetically well understood organism. Similar modifier effects are suspected for human cells in culture as well. Variations in radiation sensitivity of 'normal' cells as well as cells from ataxia telangiectasia patients have been observed (Fig.5), but in mammalian cells, techniques have not yet been established which can differentiate between leaky alleles of the same locus, or modifiers at different loci, and, for that matter, the same phenotype encoded by different loci can only be distinguished with considerable genetic manipulation. A case can be made for any of these three alternative possibilities for the variation seen in Fig.5.

This leaves us with a large number of genetic loci (possibly 200 or more) that can affect or directly alter the sensitivity to mutagens and/or the spontaneous mutation rate. From an evolutionary point of view then, there is great room for individual variation, and, except for monozygotic multiple births, it seems unavoidable that each individual must have a unique spontaneous mutation rate. Large populations tend to grow from small tribes. After a short period of stabilization of gene frequencies through genetic drift, the tribes grow in size, and accept some new gene influx, but hardly enough to alter the composition of the basic gene pool. With a large number of genes being recombined during the early history of a population, it would be expected also that the population means of the spontaneous mutation rates would differ from one another.

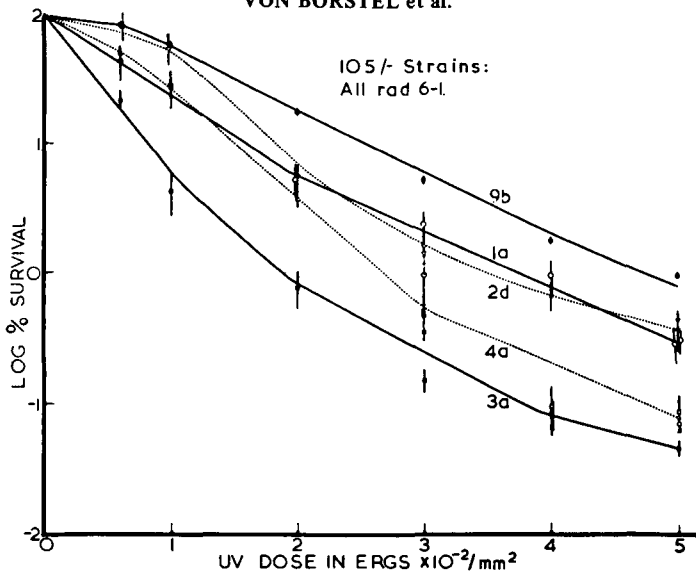


FIG. 4. Comparison of survival to ultraviolet radiation of the same radiation-sensitive allele of rad 6 in five different spore stocks from the same parent (from [28]).

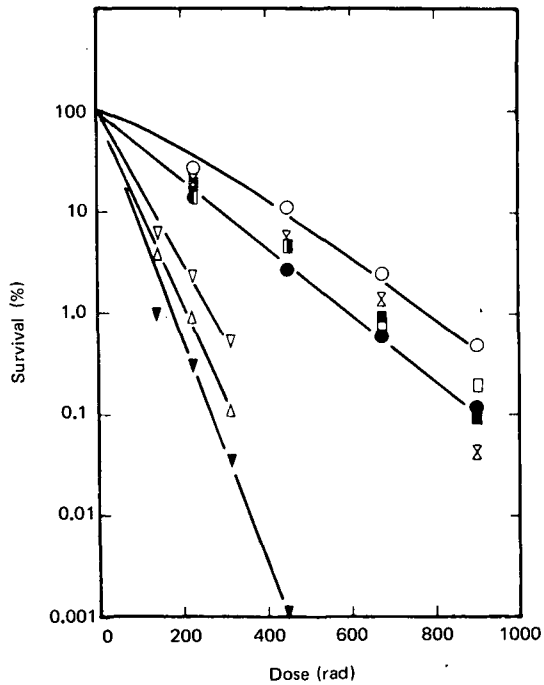


FIG. 5. Gamma-radiation response of cultured human fibroblasts. The open and filled circles designate cells from normal controls. The squares and double triangles designate cells from individuals with the basal cell naevus syndrome. The open and closed triangles designate cells from individuals with ataxia telangiectasia (from [29]).

It is necessary then to establish the mutagen sensitivities and spontaneous mutation rates of individuals and of populations before doubling doses and the hazards of mutagens can be worked out with certainty.

MEASUREMENT OF SPONTANEOUS MUTATION RATES

Precise methods have been developed to measure spontaneous mutation rates in micro-organisms [30] which can be used also to measure sensitivity of growing cells to mutagens. Counterpart studies have been carried out with primary cell lines of humans as well [25, 31], but these are difficult and expensive, and cannot be done routinely by clinical testing laboratories. An approach to measuring spontaneous mutation rates by a rapid autoradiographic method has been begun by Strauss and Albertini [32]. By taking lymphocytes from peripheral blood, stimulating them to incorporate ^3H -thymidine with phytohaemagglutinin, and then placing 6-thioguanine in the medium, it was found that only cells which were resistant to 6-thioguanine would take up the labelled thymidine. So far, Strauss and Albertini have used this method only to note the rise in frequency of thioguanine-resistant lymphocytes in the peripheral blood of cancer patients who have been treated with cytotoxic agents; potentially this or a similar method could be used to measure spontaneous mutation rates in human cells. Rapid methods are also needed for measuring rates of spontaneous and induced carcinogenic transformation of human cells that could be used as an indicator of the susceptibility to cancer of an individual. Approaches are being made to this problem [33] but we have far to go.

After rapid and accurate methods have been established for measuring spontaneous events, we then need to ascertain quantitatively the sensitivity of cells to a battery of mutagens in a way that would define defects in DNA repair as well as inform us of the presence or absence of activation systems.

Only then can a beginning be made towards defining the risk of an individual to exposure to mutagens in precise quantitative terms.

PRACTICAL USE OF THE DOUBLING DOSE

Since each individual is likely to have a unique spontaneous mutation rate, then each individual is likely to have a doubling dose for a mutagen which is also unique. Let us suppose that one individual has a spontaneous mutation rate ten times higher than another. Consequently, the doubling dose of the 10X individual would be ten times that of the 1X individual if the mutation rate increases linearly. This raises the problem that with the present doubling dose concept, the 10X individual should suffer less risk to ten times the dose given to the 1X person.

Depending on the source of the increased mutation rate, the reverse is more likely to be true. The person with the 10X spontaneous mutation rate should be more carefully protected. A person with xeroderma pigmentosum should be protected from the sunlight, not exposed to more sunlight just because he expects a higher cancer incidence anyway.

Thus the rate-doubling dose concept has a built-in logical fallacy. The doubling dose only works if we assume that the spontaneous mutation rate is the same for everyone. But despite this limitation, it is convenient at this time to make the assumption of identical rates. The mean spontaneous mutation rate should be determined for different populations, and this should be used to determine the doubling dose. Exceptions found in a population will have to be given special consideration, assigned risk indices, and the individuals concerned must be advised on how to avoid the hazards that affect them. Eventually a risk index can be assigned not just to the exceptions, but to each individual in the population. When this has been done then the doubling dose concept for populations will have served its purpose.

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DISCUSSION

K.H. CHADWICK: How 'spontaneous' is the spontaneous mutation rate? Is it constant in time?

R.C. VON BORSTEL: First we must know the proportion of the spontaneous, or better, the 'natural' mutations which come from the environmental factors that assault us. If these factors are 'increasing' rapidly, as appears to be the case, the natural mutation rate will appear to be 'increasing'. The selection for individuals in a population with high inborn spontaneous mutation rates proceeds slowly, but does indeed proceed in rapidly changing environments.

K.H. CHADWICK: I was thinking of the incidence of birth defects in the natural human population which seems to have increased between the earlier and the latest surveys. I wonder if any increase in environmental agents might be responsible for this.

ASSESSMENT OF DOSE-RESPONSE RELATIONSHIP IN CARCINOGENESIS FOLLOWING LOW RADIATION DOSAGE

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Abstract

ASSESSMENT OF DOSE-RESPONSE RELATIONSHIP IN CARCINOGENESIS FOLLOWING LOW RADIATION DOSAGE.

The quantitative experimental study of low-level radiation carcinogenesis entails numerous complex problems associated with: (1) the definition of the naturally occurring tumour spectrum, (2) the small yield coupled with long latency period for tumour production or modulation following radiation dosage, (3) the definition of a threshold dose, if any. Hence, observation of large animal samples over the entire life span is required for definition of the basic (naturally occurring) and modulated (following irradiation) tumour spectrum. As a consequence, a detailed study of the spontaneous tumour incidence in the control population is essential. The data presented are based on a total of 8229 C57 Black/6M mice, of both sexes, observed over their whole life span which, under pathogen-free conditions, extended to about 1200 days. The basic tumour spectrum was defined and subsequently used as a reference system for the comparative evaluation of the tumour yield following small doses of neutrons or gamma radiation or tritiated thymidine. The experiment was aimed at providing dose-response relationships from external versus internal irradiation. The data were analysed using the Gompertzian actuarial method. The following conclusions can be tentatively drawn: (1) In control mice, a very high incidence of tumour was observed, namely 84.4% in females versus 66.7% in males. As a consequence, it would seem that the whole concept of tumour induction versus natural tumour incidence should be re-evaluated. (2) Modulation of the basic tumour spectrum following irradiation appeared to be, in some instances, not only quantitative but also qualitative in nature, as evidenced by an observed shift from type A to type B reticulum cell lymphoma in irradiated mice at all dose levels. (3) In age-specific incidence rates for lymphocytic lymphomas, the time shift of observed values was twofold, towards relatively higher incidence earlier in life and towards relatively lower incidence in late survivors. These observations suggest an in-depth re-evaluation of some current concepts on the modulation of tumour incidence by carcinogenic agents, from the point of view of their qualitative significance and quantitative assessment.

1. INTRODUCTION

Among the late biological sequellae of ionizing radiation, none has probably been more investigated than the carcinogenic effect. For three quarters of a century, epidemiological and experimental data have accumulated from observation in humans as well as experimentation in animals. The analytical and systematic study of the correlation between radiation-induced carcinogenesis and the magnitude of dose was initiated at a much later period, using as the biological end-point the lymphocytic lymphoma in C57 Black mice, a tumour type that has been extensively investigated [1, 2]. The design and statistical treatment of a suitable experiment aimed at assessing quantitatively such a dose-response relationship in the mid-lethal and sublethal dose range was pioneered by Kaplan [3]. Later, the interest of investigators focused on late effects observed throughout the lifespan following exposure to low-level radiation, for an obvious reason, namely the need to evaluate the carcinogenic hazard for mammals (including man) exposed to small occupational or accidental radiation dose, especially in an attempt to define the risk-benefit of medical and industrial applications of nuclear energy. The quantitative assessment of the carcinogenic potency of low-level radiation raises several complex problems, among which are (1) the selection of a reproducible and reliable end-point, taking into account the spontaneous neoplasia in the experimental animal, i.e. the definition of the naturally occurring tumour spectrum, (2) the critical evaluation of the biological parameters associated with a small tumour yield, coupled with long latency periods for modulation of the natural or basic tumour spectrum as a result of irradiation at low dosage.

2. MATERIALS AND METHODS

The data presented here originated from an extensive experiment aimed at investigating the comparative late effects from fast neutron or cobalt-60 external irradiation versus tritiated thymidine internal irradiation [4]. The whole investigation required a total of 8229 mice, of both sexes, either sham-, neutron- or cobalt-irradiated at the age of 33 ± 3 days, or injected with tritiated thymidine at birth. All mice originated from pathogen-free C57 Black, subline 6 breeders purchased in 1963 from J.C. Kile, Cumberland View Farms, Clinton, Tennessee. The colony has been continuously maintained in our animal quarters for 15 years at the Laboratory of Radiobiology of the Free University of Brussels, Belgium. Fast neutron irradiation was performed under a Euratom contract, at I.T.A.L., Instituut voor Toepassing van Atoomenergie in de Landbouw, Wageningen, The Netherlands. The main characteristics of the I.T.A.L. reactor were as follows: 100 kW, swimming-pool type; ^{235}U content: 4032 g, 90% enriched; irradiation room: 2 m² field.

TABLE I. EXPERIMENTAL DESIGN (NEUTRON IRRADIATION)

<i>Control</i>		Number of mice autopsied ^a
Male		231
Female		196
<i>Irradiated with neutrons</i>		
3.2 rads	Male	143
	Female	129
4.5 rads	Male	117
	Female	122
6.3 rads	Male	112
	Female	112
8.8 rads	Male	95
	Female	94
12.3 rads	Male	79
	Female	104
17.2 rads	Male	97
	Female	77
24.1 rads	Male	60
	Female	42
33.7 rads	Male	66
	Female	49
47.2 rads	Male	44
	Female	46
TOTAL		2015

^a Dead mice from accidental death or not suitable for microscopic examination not included.

The irradiation of mice was carried out at 5 kW. Exposure times varied from 5.88 to 23.00 minutes, depending on dose. A logarithm dose gradient was selected, using a multiplying factor of 1.4, yielding the following dose levels for neutrons, 3.2, 4.5, 6.3, 8.8, 12.3, 17.2, 24.1, 33.7 and 47.2 rads. Neutron dose measurements were carried out using an acetylene equivalent ionization chamber in the mouse set-up. Gamma radiation was performed with a Picker 2000 Ci cobalt-60 therapy unit. Source-target distance was adjusted so that duration of exposures matched parallel exposures to fast neutrons. Matching gamma doses were 18, 25, 36, 51, 71, 100 and 141 rads. Gamma radiation dosimetry of

TABLE II. EXPERIMENTAL DESIGN (COBALT IRRADIATION)

<i>Control</i>		Number of mice autopsied ^a
Male		228
Female		201
<i>Irradiated with cobalt</i>		
18 rads	Male	380
	Female	385
25 rads	Male	389
	Female	372
36 rads	Male	365
	Female	373
51 rads	Male	259
	Female	240
71 rads	Male	157
	Female	156
100 rads	Male	122
	Female	128
141 rads	Male	107
	Female	71
TOTAL		3933

^a Dead mice from accidental death or not suitable for microscopic examination not included.

cobalt-60 was carried out using a Victoreen thimble ionization chamber with Lucite cap. Absorbed dose measurements were obtained from a phantom consisting of a 30-ml Lucite cylindric bottle filled with ferrous sulphate dose meter solution. Mice were irradiated in a circular Perspex container divided into radial compartments, rotating under the neutron flux or the cobalt beam. During neutron irradiation, the container was protected from gamma rays by a 5-cm thick lead shield and from slow neutrons by a 5-cm-thick lithium fluoride screen. From each litter, juvenile mice of either sex were randomly assigned to control and treatment groups, irradiated at one of the dose levels from a single exposure to neutrons or cobalt (Tables I and II). New-born mice from contemporary litters aged less than 24 h received a single intraperitoneal injection of tritiated thymidine at one of the following dose levels, 0.3, 0.4, 0.6, 0.9 or 1.5 μ Ci (Table III); Mice were allowed to live out their entire life span and upon death were promptly

TABLE III. EXPERIMENTAL DESIGN (TRITIATED THYMIDINE ip)

<i>Control</i>		Number of mice autopsied ^a
Male		178
Female		205
<i>Injected with tritiated thymidine</i>		
0.3 $\mu\text{Ci/g}$	Male	56
	Female	40
0.4 $\mu\text{Ci/g}$	Male	55
	Female	44
0.6 $\mu\text{Ci/g}$	Male	21
	Female	22
0.9 $\mu\text{Ci/g}$	Male	31
	Female	24
1.5 $\mu\text{Ci/g}$	Male	36
	Female	47
TOTAL		759

^a Dead mice from accidental death or not suitable for microscopic examination not included.

autopsied. Representative tissue samples were processed for microscopic study. Tissues were stained with haematoxylin and eosin routinely and PAS stain was used occasionally.

3. EXPERIMENTAL RESULTS

3.1. Lymphomas

Lymphomas (thymic or non-thymic) have been divided into mature and immature forms. The cells of the lymphomas were small, monomorphic, diffusely infiltrating, resembling quite closely the normal cell. As tumour masses appeared, lymphoid cells remained free from each other (non-cohesive). At autopsy the spleen, thymus and/or lymph nodes or clusters were enlarged and appeared soft and white with small haemorrhagic areas. Other organs or tissue involved later in various degrees were the kidneys, ovaries, liver, adrenal and salivary glands,

lungs and mesenteric fat. In advanced cases the bone marrow was replaced by lymphoid cells, and often adjacent bone was destroyed and muscle was invaded. Mature forms occurred with overwhelming frequency. Immature forms were occasionally found. In fact, clear-cut lymphoblastic types were so few, and the transition from the immature to the mature form so gradual, that we could not decide upon a useful and legitimate boundary between them. Thymomas were rarely observed.

3.2. Reticulum cell lymphomas

The monocytic, also called the histocytic [5] or type A of Dunn form, was the most frequently found reticulum cell sarcoma in control mice. These neoplasias were composed of a variety of cell types. Within any one animal the tumour was monomorphic and cohesive, though there could be considerable variation in cell size. The tumour mass was always organoid and displayed little or no diffuse infiltration. It expanded and destroyed surrounding tissues as a mass. The liver, uterus and ovaries were most commonly involved, but extension to the spleen, lungs, kidneys and thymus was observed on rare occasions. Ascites was invariably present. The bone marrow was rarely and sometimes questionably involved.

The Hodgkin's-like, or type B of Dunn, was the least common type of the reticulum cell tumours in control mice. The striking characteristics of this neoplasm was its pleomorphic array of cells. Cell types ranged from lymphocytes to reticulum cells to giant multinucleated cells. Frequently the reticulum cell component would contain much ingested debris. The cells were incohesive, organoid masses, and spread by massive, tissue-destroying frontal infiltration.

In irradiated mice, at all dose levels, there was a shift from type A to type B reticulum cell lymphomas. Whereas the former appeared very difficult to identify and was infrequently encountered, the latter was observed with overwhelming frequency.

3.3. Hepatomas

Hepatomas ranged from small nodules that escaped detection at autopsy to large overwhelming masses that obscured the normal structure of the liver. The small tumours usually were seen microscopically as sharply delineated groups of cells which would interrupt the usual pattern of the liver cells. The tumour cells were often not greatly different from the hepatocytes except for their enhanced size, but did show some degree of pleomorphism. The large tumours, on the other hand, had a loss of lobular pattern and rarely, if ever, could portal triads or bile ducts be observed. The blood supply of the tumour of the large variety was apparently from hepatic arterioles. Some tumours were composed

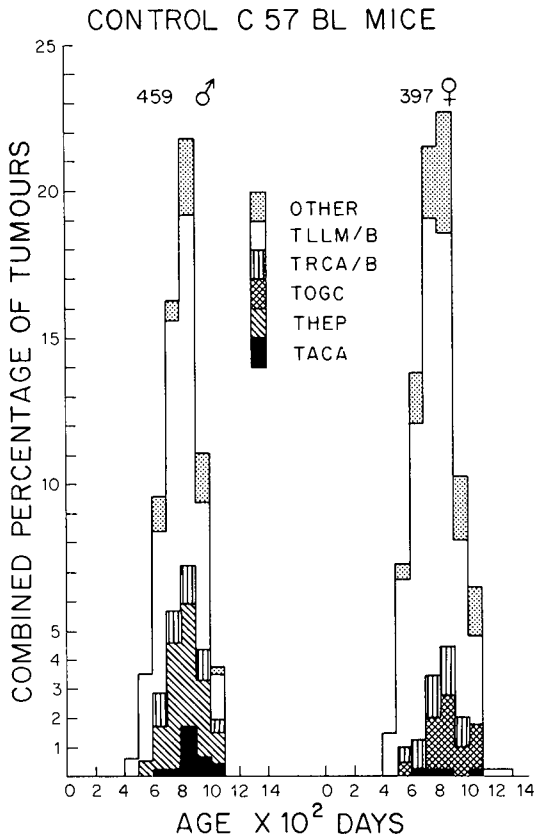


FIG.1. Basic tumour spectrum in male and female control mice.

Tumour coding system

- TLLM/B:** Tumour lymphocytic lymphoma mature (a few immature B forms are included).
No separation of thymic and non-thymic forms.
- TRCA/B:** Tumour reticulum cell form A (a few B forms are included).
- TOGC:** Tumour ovary granulosa cell.
- THEP:** Liver tumours – malignant, benign and nodular hyperplasia of hepatocytes combined.
- TACA:** Alveologenic tumour of the lung (Stewart classification) malignant or benign.

of strands or cords of tumour cells separated by large blood-filled pools. Others were large sheets of thickened cords. Still others were composed of tube-like structures with variably sized bile canaliculi. No bile pigments were seen in these tubes.

Amyloid was on occasion seen in the tumour, most particularly if the deposits were also seen in the normal parts of the liver. In addition, lympho-sarcomatous collections in the tumour mass would often be seen. Proliferation of bile ducts was quite uncommon and never severe in extent.

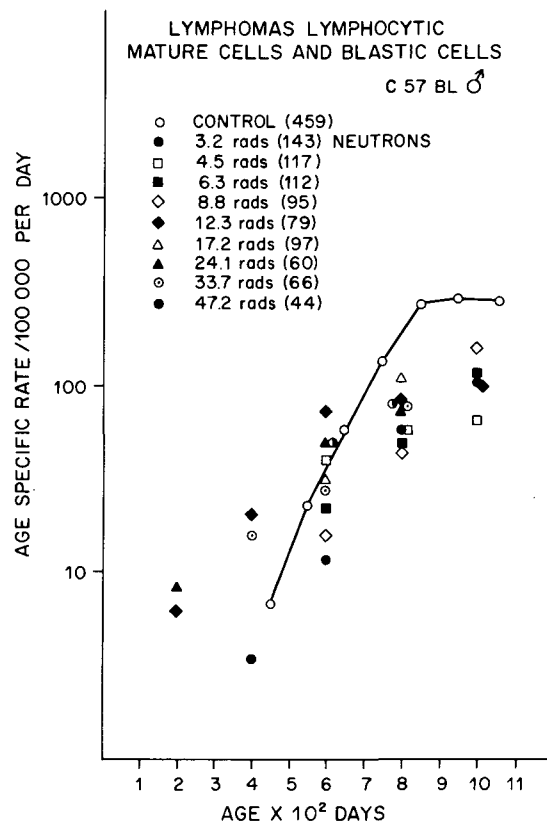


FIG.2. Age-specific incidence rates for combined mature and immature lymphocytic lymphomas in male mice following neutron single exposure.

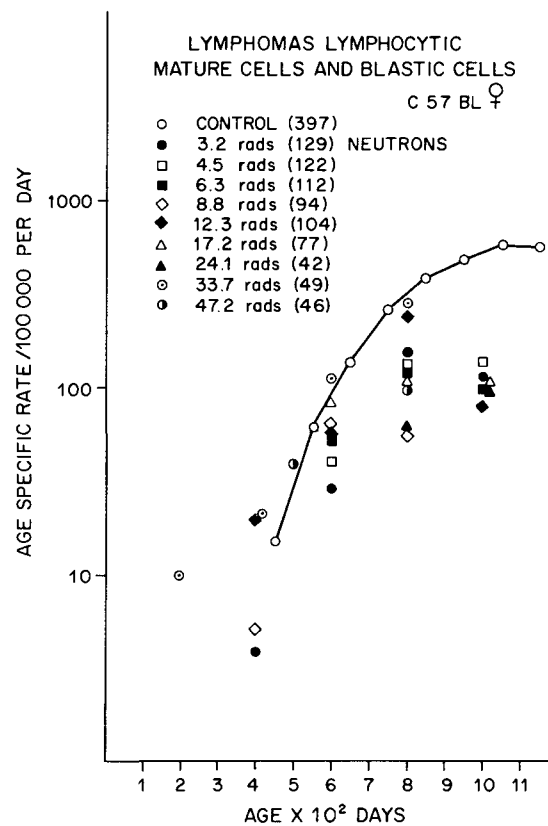


FIG.3. Age-specific incidence rates for combined mature and immature lymphocytic lymphomas in female mice following neutron single exposure.

3.4. Oncogenic spectra in control male or female mice

Figure 1 shows the combined percentage of all tumours in histogrammatic distribution over age in male and female control mice. As a rule, female animals experience a higher tumour incidence than males, 84.4% versus 66.7%. This percentage refers to all observed tumours within all animals at risk, i.e. that were followed up throughout their entire life span and underwent complete autopsy following 'natural' death.

A second feature is the higher incidence of lymphomas in females (57.9% versus 36.4%) which accounts for most of the variation in the overall tumour incidence in either sex. As stated above, mature forms were overwhelmingly more frequent than immature. Both forms were combined for statistical analysis. Similarly in reticulum cell tumours, which accounted for 4.7% and 4.6% of tumour incidence respectively in male and female mice, type A largely outnumbered type B forms. Again, both forms were combined for statistical analysis.

It is noteworthy that the incidence of a sex-specific neoplasia, namely the granulosa cell tumour of the ovary in females (7.6%), was largely offset by the apparently selective occurrence of liver tumours, including hyperplastic nodules, in control males (14.6% versus none in females). Part of the balance was further taken up by the higher incidence of alveologenic tumours of lung in males (3.3%) in contrast with females (0.7%). Other tumours were diversely distributed among sexes with higher overall incidence in females, but no clear sex-linked abundance, probably because of small sample sizes. Other observed tumours were various adenocarcinomas, squamous cell carcinomas and leiomyosarcomas of the gastrointestinal tract, liver and spleen haemangiomas. Micro- and macrofollicular hyperplasia, as well as papillary adenomas, of the thyroid were noted. Fibromas and fibro-sarcomas of connective tissue, muscles and bones, myomas and fibro-sarcomas of uterus, luteomas and cysts of the ovary, papillary and transitional cell carcinomas of kidney and an occasional mammary adenocarcinoma or skin epithelioma were observed.

3.5. Modulation of mice tumour spectra following neutron or cobalt external irradiation

Age-specific mortality rates for specific causes of death are derived in the following manner. The death rate for a given cause, r_s , is

$$r_s = \ln \left(\frac{N_i}{N_i - n_i} \right) / t_i$$

where n_i is the number of animals dying from a specific cause within the age interval of t_i days, in a population of N_i survivors at the beginning of the interval.

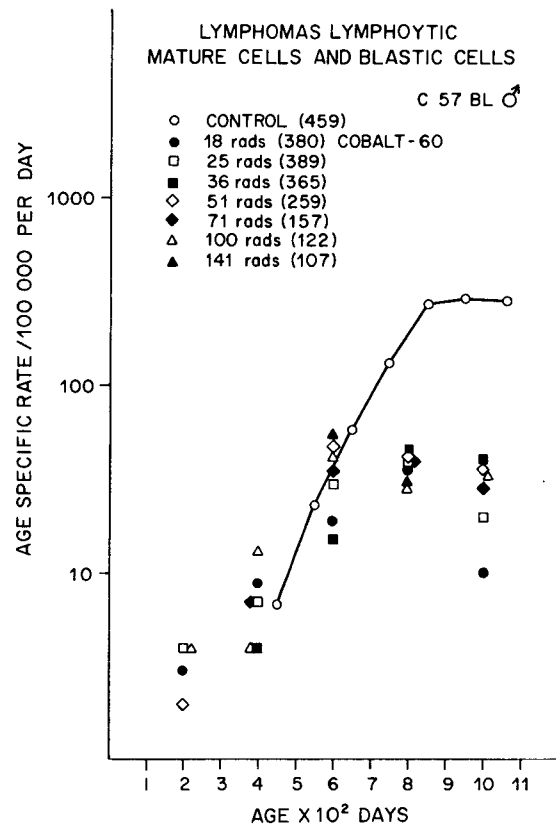


FIG.4. Age-specific incidence rates for combined mature and immature lymphocytic lymphomas in male mice following cobalt single exposure.

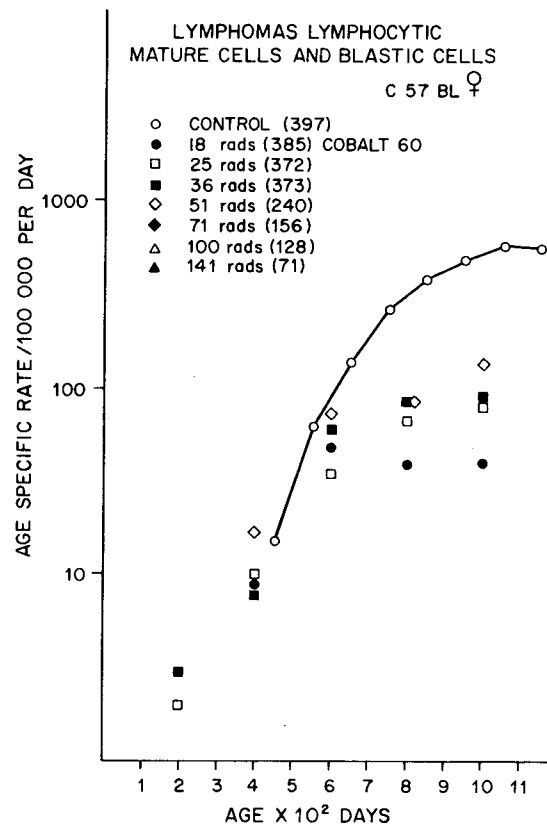


FIG.5. Age-specific incidence rates for combined mature and immature lymphocytic lymphomas in female mice following cobalt single exposure.

Age-specific incidence rates for combined mature and immature lymphocytic lymphomas respectively in male and female mice following neutron irradiation are shown respectively in Figs 2 and 3. The lymphocytic lymphoma incidence was consistently higher in females than in male control mice within all age groups, and it is obvious that a specific sex-linked susceptibility also existed in irradiated animals. However, age-specific rates tended to approach a similar trend both in female and male irradiated mice, by reaching parallel average values within matching age intervals. The time shift of observed values was twofold, towards relatively higher incidence earlier in life and towards relatively lower incidence in late survivors, that is, a negative time shift was induced during the first half of the life span and was balanced by a positive one during the second half.

Age-specific incidence rates for combined mature and immature lymphocytic lymphomas respectively in male and female mice following cobalt irradiation are shown in Figs 4 and 5. The cobalt curves exhibited, to a lesser degree, basic features similar to those observed in the neutron curves. In particular, a twofold balanced time shift was again apparent.

Age-specific incidence rates for reticulum cell lymphomas respectively in male and female mice following neutron irradiation are shown in Figs 6 and 7. The reticulum cell lymphoma incidence was slightly higher in female than in male control mice between the 600 and 1000 day interval. However, a marked sex-linked susceptibility appeared in irradiated animals, female animals being strikingly more susceptible than male.

Age-specific incidence rates for reticulum cell lymphomas respectively in male and female mice following cobalt irradiation are shown in Figs 8 and 9. Again a much higher susceptibility appeared in female irradiated mice.

It is noteworthy that in none of the neutron or cobalt irradiated groups the age-specific incidence of reticulum cell lymphomas exhibited a composite balanced time shift. The latter was consistently negative in all instances, regardless of sex.

3.6. Modulation of mice tumour spectra following administration of tritiated thymidine

Table IV shows the variation of tumour incidence in both male and female recipients of tritiated thymidine versus control animals. It is seen that the overall tumour incidence was similarly increased in both sexes. The observed increments were statistically significant by the chi-square test at the 0.05 level. The overall increase in crude tumour incidence appeared to be attributable to enhanced lymphosarcoma incidence. Fluctuations in incidence of other tumours were not statistically significant.

Figures 10 and 11 show the age-specific tumour incidence rates for tumours of reticular tissue respectively in male and female controls versus animals injected with tritiated thymidine. Most incidence rates for injected mice are higher than corresponding control rates.

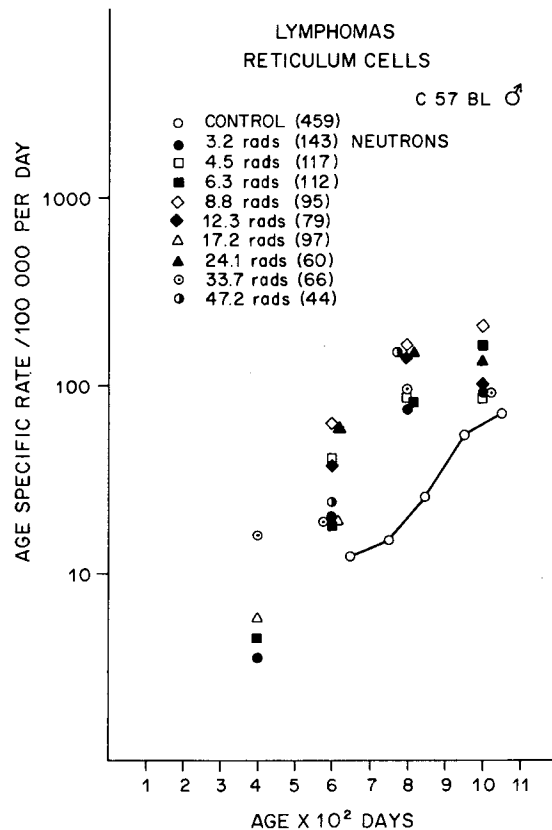


FIG. 6. Age-specific incidence rates of reticulum cell lymphomas in male mice following neutron single exposure.

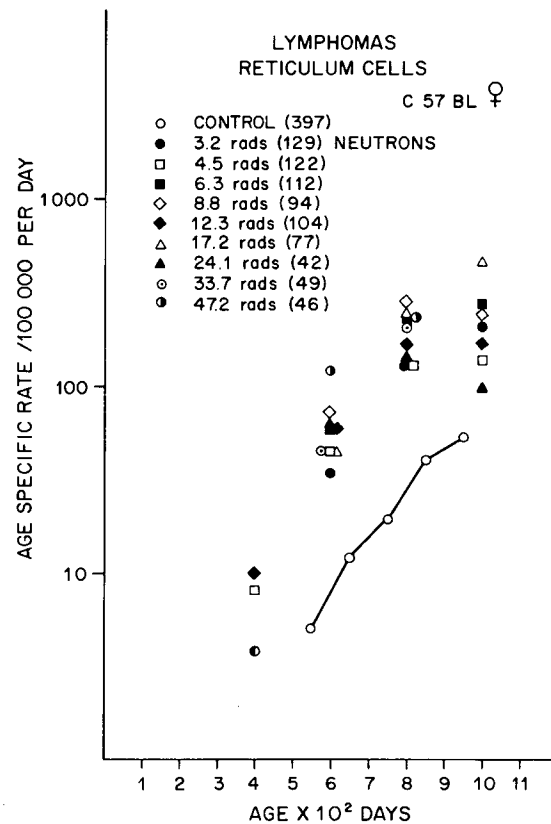


FIG. 7. Age-specific incidence rates of reticulum cell lymphomas in female mice following neutron single exposure.

TABLE IV. VARIATION OF TUMOUR INCIDENCE IN PERCENTAGE IN TRITIATED THYMIDINE RECIPIENTS (344)

	Mice (male)	Mice (female)	P
Lymphosarcoma	+9.4	+9.3	0.05
Thyroid	-1.4	-0.6	
Liver	-0.1	+0.1	
Lung	+1.7	-4.0	
Others	-3.2	-1.3	
All tumours	+4.9	+5.2	0.05

No statistically significant dose-response relationship could be derived for tumour incidence rates between different dose levels in groups of mice injected with tritiated thymidine, mainly because of small sample sizes.

3.7. Relative time frequency of hepatomas, alveologenic adenomas and granulosa cell tumours following neutron or cobalt external irradiation

In Figs 12 and 13, frequency distribution of hepatomas with age are presented for male and female mice, unirradiated and irradiated, at selected dose levels. It is seen that in males no dose-related time shift of latency periods occurs whereas the yield seems related to dose, at least above the 18-rads dose level. In females, the increased yield resulting from irradiation is obvious since the control incidence was nil. There is also a suggestion that the latency periods may be shortened in irradiated groups.

In Fig. 14, the dose-related incidence of alveologenic adenomas in male and female mice and of granulosa cell tumours in females is presented. The striking feature for lung tumours is that the low female incidence is abruptly brought upward to the male incidence level by any amount of radiation. The granulosa cell tumour incidence, on the other hand, seems to exhibit very little dose effect, if any.

3.8. Tumour-host/animal and tumour/host index

Tumour incidence, when expressed in terms of total number of tumours over total number of animals at risk in any one group, overlooks the factor of tumour multiplicity in the tumour-bearing animals. Therefore it seems interesting

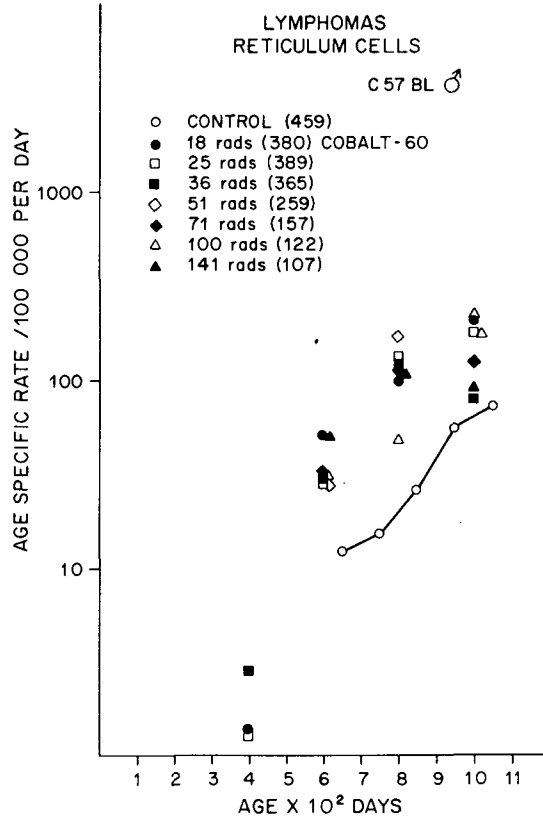


FIG.8. Age-specific incidence rates of reticulum cell lymphomas in male mice following cobalt single exposure.

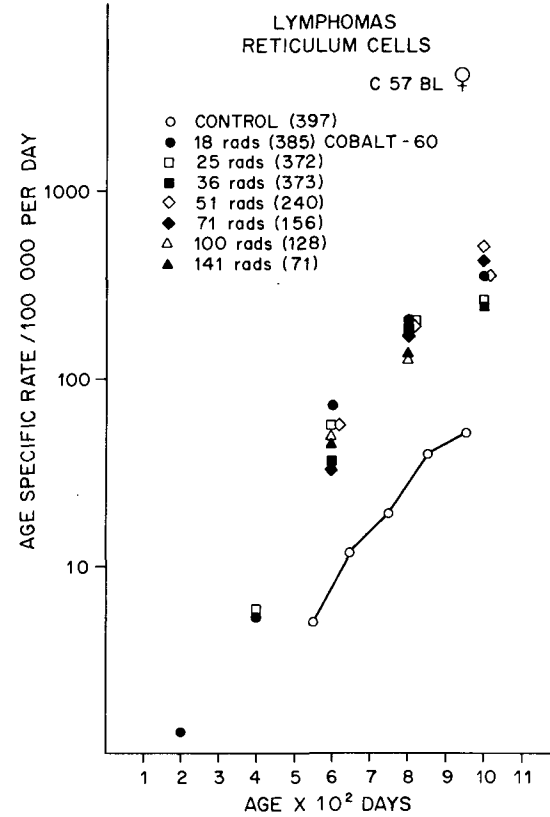


FIG.9. Age-specific incidence rates of reticulum cell lymphomas in female mice following cobalt single exposure.

to correlate with dose the frequency of tumours expressed per host as distinct from the frequency per animal at risk. We define it as the tumour per host index or tumour/host index. Figure 15 shows the tumour/host index compared with the tumour-host per animal index, that is the ratio of tumour-bearing animals to all animals at risk. In either sex, both fall sharply then subsequently increase with dose.

4. DISCUSSION

4.1. Tumour incidence spectra

We have previously reported that C57 Black/6M mice exhibit during their lifespan a large spectrum of tumours. About 20 years ago, this mouse strain was classically described as a relatively tumour-free line, except for lymphocytic lymphoma and reticulum cell sarcoma [6]. Nowadays, it has been shown to produce almost all known types of murine tumours [7]. No exogenous or environmental carcinogenic stimulus is apparently required as control mice are confined in cages maintained in a controlled environment devoid from known pollutants or carcinogenic agents. In our mice colony the observed overall tumour frequency has substantially increased over the last 15 years, apparently not from some hypothetical oncogenic variation but rather (a) because more anatomical sites were explored where tumours were observed *in situ*, and (b) because more tumours developed in various sites as mice life span was greatly increased through improved conditions of maintenance. As an example, systematic examination of larynx and related structures disclosed an unsuspected frequency of thyroid tumours. A further systematic search involving serial sections of other organs would most probably bring about an even larger tumour spectrum of greater diversity. The limiting factor, as we see it, has simply to do with our ability and willingness to devote the time, money and effort to examine exhaustively more of the autopsy tissue material.

It should be borne in mind that many tumours exhibit their own age-specific peak incidence within a fairly well defined time distribution [8]. As a consequence, the basic tumour pattern, as evidenced by actuarial analysis, appears more likely to proceed from inter-competitive stochastic processes than from some probabilistic random distribution. In other words, the age-specific incidence of any particular tumour may actually depend upon the contingent age-specific incidence of one or several concomitant tumours. In our opinion, it would be misleading to dissociate any particular tumour type from the entire control or modulated tumour pattern as a whole, in an attempt to use it as an end-point for evaluation of radiation effects. This is particularly important in dealing with low dosage radiation resulting in limited multivariate shifts of tumour spectra even within

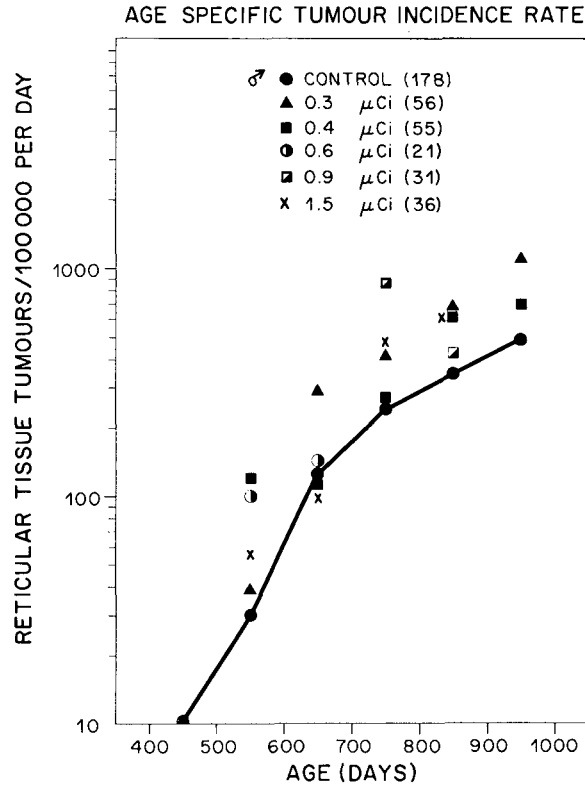


FIG.10. Age-specific tumour incidence rates for reticular tissue tumours in male control versus male experimental animals injected with tritiated thymidine at any dose level.

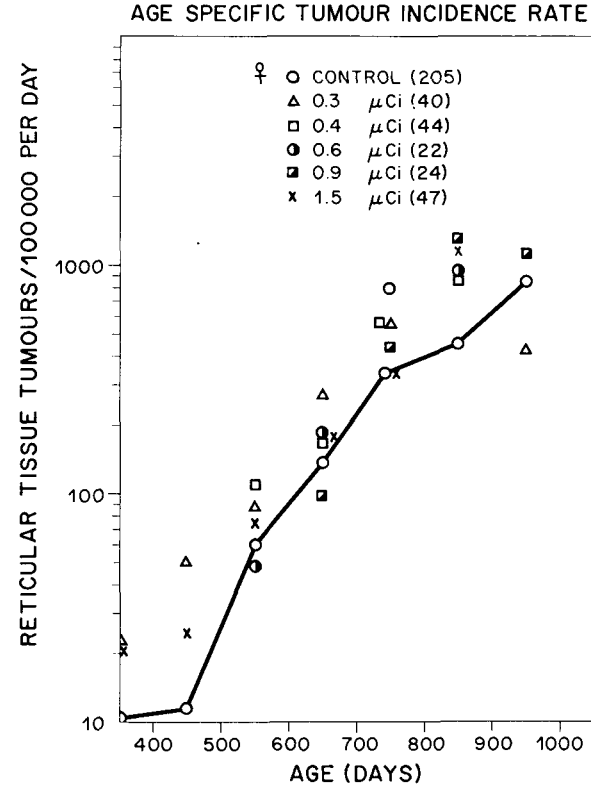


FIG.11. Age-specific tumour incidence rates for reticular tissue tumours in female control versus female experimental animals injected with tritiated thymidine at any dose level.

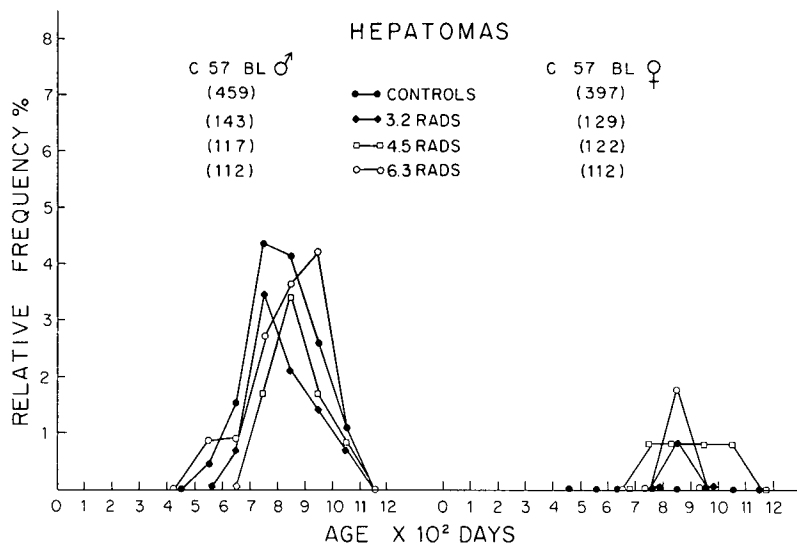


FIG.12. Frequency distribution of hepatomas in male and female, control or irradiated mice.

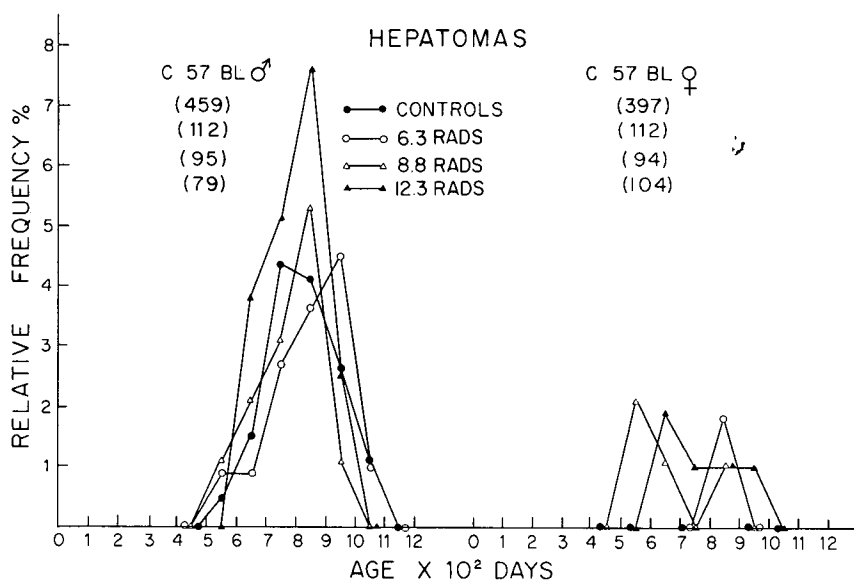


FIG.13. Frequency distribution of hepatomas in male and female, control or irradiated mice.

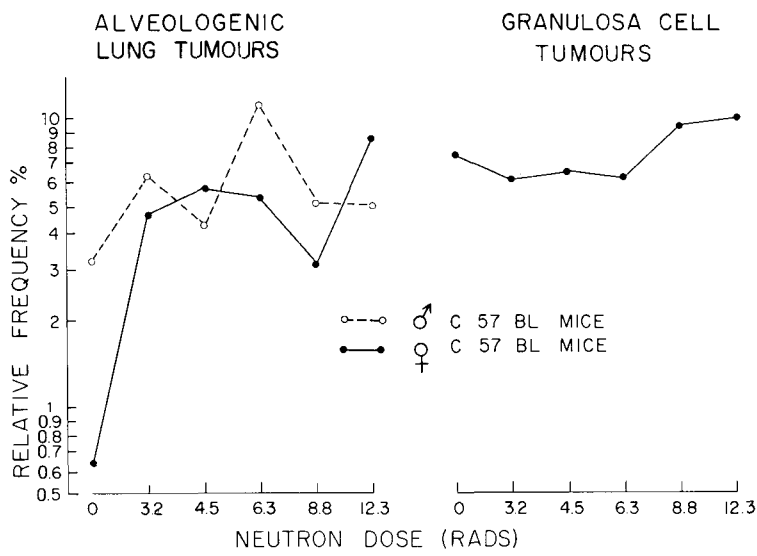


FIG.14. Dose-related incidence of alveologenic lung tumours and granulosa cell tumours in control or irradiated mice.

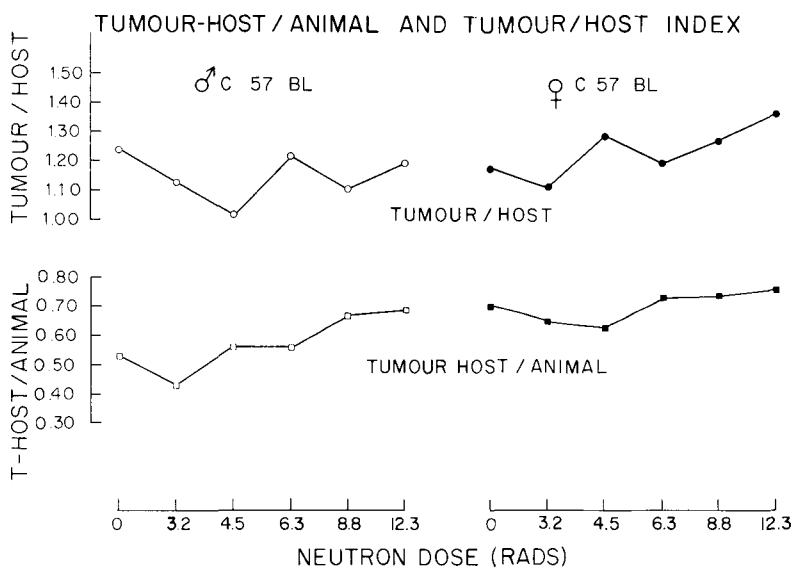


FIG.15. Frequency of tumours (all types) per tumour-bearing animal (host) and frequency of tumour-bearing animals (host) per animal at risk, as related to dose.

fairly large population samples of mice. In such instances the quantitative analysis of tumour production obviously entails numerous uncertainties which usually become minimized in the event of a predominant process such as, for instance, the increased incidence of some specific tumour type from a negligible to a substantial level, with minimal shift of the basic tumour spectrum following irradiation in the sublethal dose range.

4.2. Dose-response relationships following single external or internal exposure

In attempting to derive dose-response relationships from the data reported here on C57 Black mice, one is confronted with the diversity and multiplicity of the basic tumour spectrum. This feature is in itself a major problem. The high frequency of spontaneous tumours, averaging 84% and 67% respectively in female and male animals, raises the problem of selecting a reliable and significant end-point that could be used as a reference standard for assessing the potency of any exogenous carcinogenic stimulus. There seems to be no single parameter that would qualify as a random variable.

As far as the dose (the 'independent' variable) is concerned, no attempt will be made here to discuss the implications and limitations for intercomparison of external gamma or neutron irradiation single doses versus internal continuous exposure from tritium, with respect to the distribution of absorbed energy, the R.B.E. and its dose dependence and other quantitative factors including sublethal repair of damage. It is our endeavour, at this time, to give consideration in some depth to the biological end-point, i.e. the observed tumour incidence. It seems almost trivial to make the statement that the qualitative as well as quantitative assessment of the response (the 'dependent' variable) will essentially rest on the observed variation of some tumour type(s) in the absence versus in the presence of the stimulus (radiation). The theoretical approach appears rather simplistic when confronted with experimental facts, and many questions can be raised in this connection. For example, is it legitimate to use the variation of *one* tumour type over a limited range of action of the stimulus? Can a particular tumour type be reasonably assumed to behave as expected from a random variable? What are the implications of a possible inter-competitive process of initiation and/or development of the tumour, which eventually can result in an apparent shift in tumour type? Answering such questions is always difficult and sometimes impossible. As a consequence great care should be exercised in selecting and evaluating any particular tumour as an end-point. Under our experimental conditions using low-level radiation delivered as single dose, the following observations have been made:

(a) The non-linearity of the response is predominantly prevalent. For example, the tumour-host/animal index and the tumour/host index tend to

decrease at lower doses whereas this tendency reverses itself at higher doses following neutron irradiation (Fig.15). The non-linearity of the response in radiocarcinogenesis has been previously emphasized by Grahn [9].

(b) The age-specific incidence rate for lymphocytic lymphomas, possibly for other tumour types, and indeed for the overall tumour incidence, in neutron- or gamma-irradiated mice, decreases with age, with respect to control animals (Figs 2 – 5). This phenomenon has been described as a negative time shift of incidence and the underlying mechanisms are poorly understood. It is in contradiction with the widely accepted hypothesis that cancer incidence rates should somehow increase steadily with age, as a consequence of a progressively less effective immuno-surveillance. On the other hand, it is in agreement with the hypothesis that in a pre-fixed proportion of animals, tumour development results from the turning-on of specific oncogenes. Accordingly, accelerated development of the tumour in the early affected hosts could prematurely deplete the cohort of predisposed animals, i.e. bearing the specific oncogenes, among all animals at risk.

(c) In contrast, the incidence of reticulum cell lymphomas seems more uniformly enhanced in neutron- or gamma-irradiated mice throughout the entire life span, with respect to control animals.

In any event, it is obvious that the age-specific spectrum of tumours constitutes a more reliable and representative end-point. Consequently, in attempting to assess dose-response relationships in carcinogenesis, the dependent variable (the response) of choice consists of the modulation of the basic tumour spectrum. This, in turn, makes it a prerequisite to define the specific basic tumour spectrum of the mouse strain used. This method certainly offers many advantages in analysing data characterized by small yield over relatively long observation times. In some instances, the most frequently observed tumour (both as spontaneous or induced neoplasia) may serve as a suitable biological end-point, provided the variation between the basic tumour spectrum and the modulated tumour spectrum appears reasonably confined to this particular tumour type. In this restricted sense, the lymphocytic lymphoma in the C57 Black strain has been widely used in the past by numerous authors and also in our studies on the carcinogenic potency of tritiated thymidine. In the latter instance, the overall increase in the overall tumour incidence appeared to result practically from lymphocytic lymphoma incidence enhancement (Table IV), whereas the variation of incidence for other tumour types did balance out. Our final results confirmed previously reported data by Lisco and co-workers [10] and by our group [11]. The reticulum cell lymphomas constitute a similar case (Figs 6–9) following low-dosage neutron or gamma irradiation.

The enhancement of reticulum cell tumour incidence appeared highly significant in C57 Black mice following neutron as well as cobalt irradiation.

This result is at variance with observations made by Upton and co-workers [12] on RF mice, following single doses of gamma radiation. In the latter strain of mice, reticulum cell sarcomas appeared to develop as a late-occurring non-radiation-induced disease. Such findings point to the genetic variability of different mouse strains, and are a warning signal against generalization or oversimplification in tumorigenesis.

It has been argued by Lindop and Rotblatt [13] that a definite correlation exists between the acceleration of a given cause of death following irradiation and its rate of change of incidence with respect to dose. They have inferred that this change of incidence is merely the result of the changed times of onset of diseases, since an animal which would have normally died of one disease will die earlier of another disease which is more accelerated by radiation. Our data are consistent with the existence of such a mechanism, and further suggest that an inter-competitive process of tumour initiation and/or development is triggered or accelerated by radiation. Some preliminary data reported by Ainsworth and co-workers [14] from an extensive investigation of late effects of neutron or gamma irradiation in hybrid B6CF1 (C57 Black \times BALB/c) mice, involving fractionated as well as single doses, seem in accordance with such a working hypothesis.

ACKNOWLEDGEMENTS

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DISCUSSION

H.H. VOGEL: In your excellent paper you mentioned that some intestinal neoplasms were also observed. In view of the well-accepted 'gastro-intestinal syndrome' in acutely irradiated mice after fission neutrons, one might perhaps expect to find a higher incidence of gastro-intestinal tumours in the neutron-irradiated mice than in gamma-irradiated mice or in their unirradiated controls. Did you find any such increase in your neutron-irradiated mice? (I realize that your highest neutron dose was less than 50 rads, and perhaps this dose wasn't high enough to severely damage the mucosa of the gastro-intestinal tract.)

D.J. MEWISSEN: At the neutron dose levels tested we did not observe any significant increase in gastro-intestinal tumours, nor did we detect any of the symptoms generally associated with the gastro-intestinal syndrome.

D. GRAHN: Were the controls contemporary with the irradiated mice? The exceptionally high upward displacement of the reticulum cell tumour rates at these low doses is as difficult to interpret as the general observation that the lymphocytic tumour death rates underrun the controls for most of the lifetime, and leads one to suspect that the controls were studied at a different time and place. Have you attempted to employ such statistical tools as a covariance analysis to test for parallelism of Gompertz slopes and for any dose-dependent displacement of the slopes from the control (in either the upward or the downward direction)?

D.J. MEWISSEN: The controls were contemporary with the irradiated animals. It is true that in long-term experiments on late effects, the possibility of a shift in the basic tumour spectrum of control animals should be kept in mind. In this respect we are currently analysing biographical data on two successive large series of control mice of both sexes. We have not as yet observed any significant shift in the respective tumour incidence spectra.

We have not used covariance analysis to test the parallelism of Gompertzian regression curves or of dose-dependent displacements, mainly because we observed non-linear regressions in tumour incidence rates with age.

V. COVELLI: Is the final incidence of reticulum-cell lymphomas statistically significant?

D.J. MEWISSEN: The relative total incidence of reticulum-cell lymphomas recorded over the life-span in neutron-irradiated animals is significantly different from the control incidence in pooled data from all experimental groups.

THE LOW-LEVEL SHAPE OF DOSE RESPONSE FOR CHROMOSOME ABERRATIONS*

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Abstract

THE LOW-LEVEL SHAPE OF DOSE RESPONSE FOR CHROMOSOME ABERRATIONS.

The existing information on dose risk at the low-dose range is derived mainly from an extrapolation of the effects of higher doses or from the effects on a certain population group whose irradiation dose had been roughly assessed. Chromosome aberrations have been investigated in the peripheral blood lymphocytes of people whose annual radiation burden was calculated and accumulated individually according to their time of stay at their respective living and working sites, whereby the external gamma irradiation as well as significant mean values of the contents of radionuclides in the atmosphere were measured at every single place. Specific organ doses were also calculated. The data show that the sum of chromosome breaks for a continuous environmental low-level irradiation rises sharply with the annual combined alpha-plus-gamma dose. According to microdosimetric considerations, dose effects in the low-dose range are mainly results of one hit damage, and dose-rate independent. An extrapolation of the low-dose slope would never meet the well-known dose-effect curve for single in-vitro doses higher than 100 rads which are found to be linear in the log-log scale. On the other hand, an extrapolation of the high-dose response curve to the low-dose range would supply aberration values that were too low. Material from the literature where doses had been given either by the authors or could be estimated from the reported data are taken into consideration. From these data, and data in literature, it can be concluded that the initial part of the dose-effect curve for chromosome aberrations is not linear or sigmoid with a threshold at the lowest dose, but rises sharply and passes into a convex upward form with a kind of plateau until it meets the linear curve of the high dose. This could be explained by postulating that at natural levels of radioactivity a basic amount of repair enzymes is present, and with increasing

* This work is supported by IAEA Research Contracts Nos 791 and 1493.

radiation aberrations increase. The stimulation of additional enzymes is induced only after a certain level of damage to the DNA, explaining the plateau effect. When this inducibility is saturated, aberration levels again rise.

1. INTRODUCTION

The effects on biological systems from ionizing radiation at very low burdens, namely of up to one or two orders of magnitude above the lowest environmental level (about 80 mrad yearly¹) are basically difficult to obtain, because of the large number of events needed to produce a statistical significance. Dose effects on living organisms beneath environmental levels are not available², and all data given in the literature at 'zero-dose' refer actually not to 'zero' but to 'environmental dose'. Estimates of radiation effects and related risks at low levels are extrapolated from curves obtained at high doses and dose rates. Experiments on biological effects in these ranges fit best to the theoretically derived relationship between effect, y , and dose, D , expressed by the equation $y = \text{const} + \alpha D + \beta D^2$, based on the theory of dual radiation action [2, 3]. This relationship plotted in log-log scale consists of a curve with two slopes whereby the change of slope is in the region of $D = \alpha/\beta$ (that dose at which the contributions of the linear and dose-squared terms are equal) (Fig. 1). Plotting this curve on linear co-ordinates, the lower part is again a straight line. Linearity in the low-dose range (shaded areas in Fig. 1), however, is not based on experimental evidence. In the historical perspective it was part of the philosophy to believe in a threshold (sigmoidal) or a non-threshold (linear) model. During the past 15–20 years persons concerned with radiation protection have used linear extrapolations to estimate radiation risks at low doses. This linear extrapolation has recently been criticized on both experimental and theoretical grounds [4, 5]. For example Baum [6] presents a convex upward dose-response curve for malignancies in the Japanese A-bomb survivors. The same shape for the dose-response curve has been demonstrated in experiments on in-vitro radiation-induced transformation of hamster embryo cells [7]. Analysis of human data on cancer risks at low doses also suggests that the effects are higher than that estimated by linear extrapolation of high doses [8].

As chromosome aberrations are considered to be the most sensitive visible biological indication of ionizing radiation, and possibly related to radiation carcinogenesis, they have been used in this paper to establish a dose-response curve. There are a large number of reports in the literature on chromosome aberrations caused by in-vitro radiation of blood at high doses (> 50 rads), but only three reports [9–11] describe the effects at lower doses, down to 5 rads.

¹ From cosmic rays, external gamma irradiation and an internal load derived from ⁴⁰K and other radionuclides.

² With the exception of investigations underground as, e.g. Ref. [1].

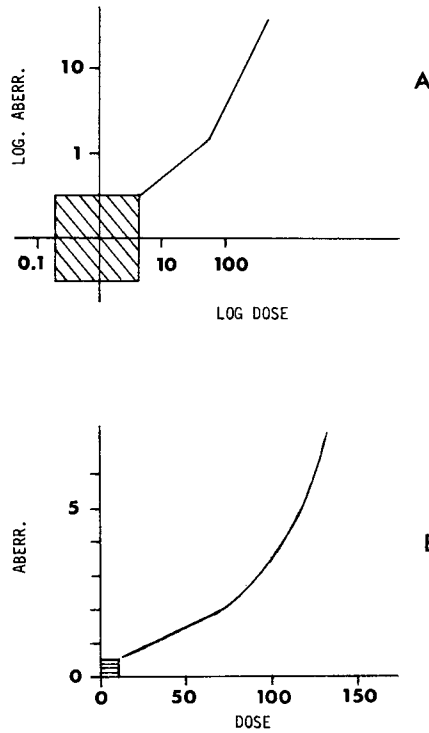


FIG.1. Dose-response curve based on the theoretically obtained equation $y = C + \alpha D + \beta D^2$, (A) log-log scale, (B) linear co-ordinates. Shaded area delineates burdens encroaching on environmental dose levels.

These have a common finding, namely that at levels beneath about 30 rads the aberration rates no longer follow the accepted dose relationship, but show an anomalous effect leading to the appearance of a plateau. This plateau is not explained by current hypotheses and, although only the result of Luchnik and Sevankaev [9] is statistically significant, the similarity between the curves presented in these three papers is striking (Fig.2).

In the dose range between 1 and 10 rads, there are several studies of chromosome aberrations in persons either occupationally or therapeutically exposed to ionizing radiation. The burdens in these investigations are given in various ways, as for example in 'working level months' (WLM)³, accumulated over years of

³ Working level (WL) is a unit for the concentration of short-lived decay products of ²²²Rn in the atmosphere, especially used for uranium mines. A worker, exposed to one WL for 167 h, receives one 'working level month' (WLM).

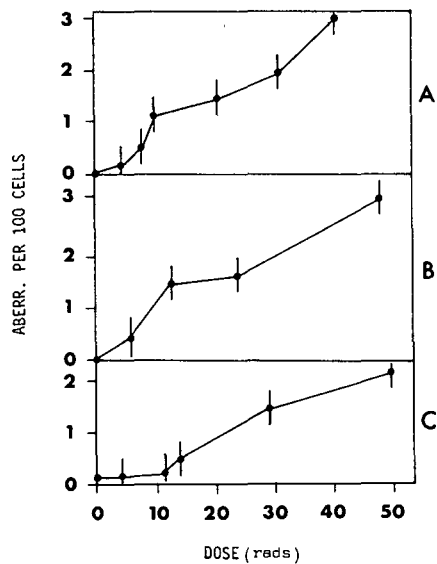


FIG.2. Dependence of the yield of chromosome aberrations in human lymphocytes upon the dose in the range 5–50 rads taken from Luchnik and Sevankaev [9]. (A) Data from Ref. [9] (gamma rays, dicentric), (B) Schmickel [10] (X-rays, all aberrations), (C) Kučerová and co-workers [11] (X-rays, dicentric and rings).

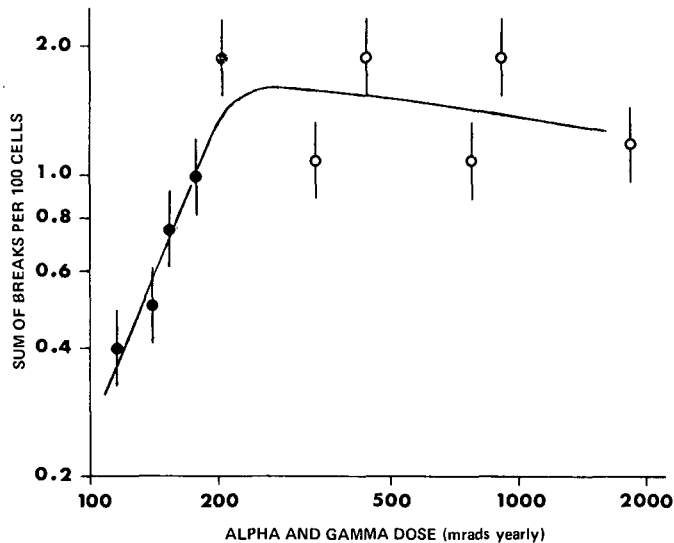


FIG.3. Results of investigations derived from a population living and working in an elevated natural radiation environment [12, 14, 19]. Each point is based on 2500 to 3500 scored cells. The errors are calculated according to a Poisson distribution.

- Persons with continual burdens mainly due to gamma irradiation
- Persons with occupational (fractionated) burdens mainly due to internal alpha irradiation.

occupation, or as the content of radionuclides as ^{226}Ra or ^{212}Pb either in the whole or in a part of the body (bone, liver, spleen). An assessment of the adequate blood dose is difficult but has been attempted (see below). In some cases the external gamma irradiation has also been given. However, all these results can only be evaluated as mean yields of aberrations for a group of persons exposed to an average load. Additionally, data on populations living in regions with elevated radiation environments are available, however here also only single points representing a mean value above the dose of normal natural radioactivity can be plotted.

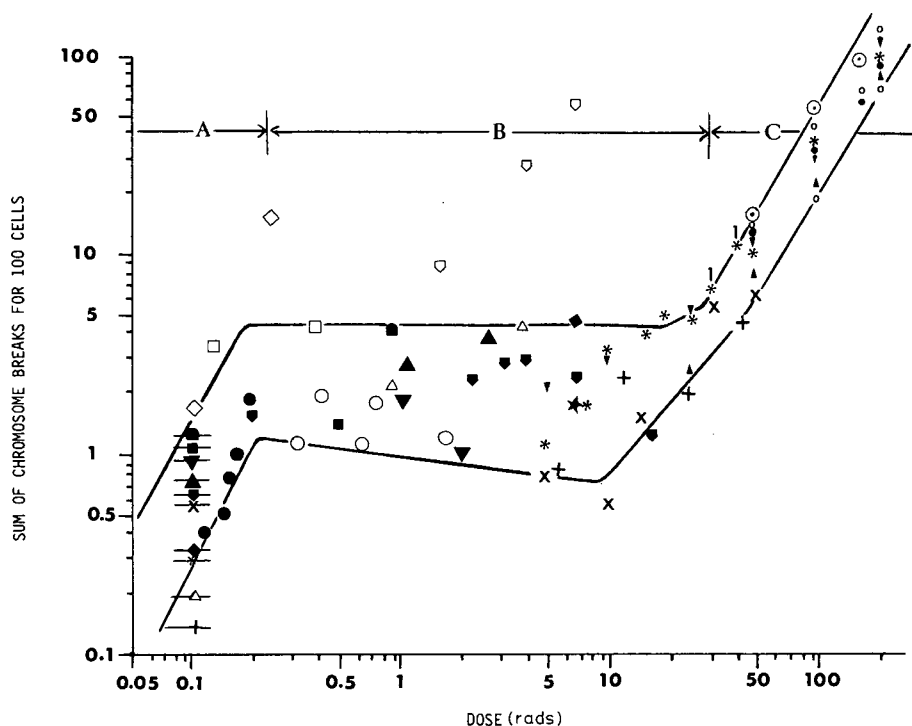
We have attempted to obtain a dose-effect curve for chromosome aberrations in the lymphocytes of the peripheral blood due to environmental and occupational gamma and alpha irradiation with accumulated 'blood doses' ranging from 80 to 260 mrad yearly for gamma and from 0.8 to 1600 mrad yearly for alpha burdens. The blood doses have been calculated for each of 122 individuals, whereby the external gamma radiation as well as significant mean values of these radionuclides in the atmosphere causing the internal alpha dose were measured for each place of the test persons stay.

Because of these individual dose calculations, our study differs from other similar investigations. Each point on our dose-effect curve (Fig.3) was obtained from 2500 to 3500 cells of 2 to 12 test persons whose radiation burdens were similar, falling within a relatively narrow dose range. The dose-effect curve rises sharply in the range of natural environmental radiation, and flattens after about 300 mrad yearly to produce a plateau which at levels corresponding to our highest burdens could even bend downwards.

The goal of this work is to confront our data with material from the literature at both low and high doses in order to attempt some conclusions concerning the initial shape of the dose-effect curve.

2. MATERIALS, METHOD AND RESULTS

The population studied by us live and work in the region of Badgastein, a 600-year-old spa in the Austrian Central Alps. The therapy of the spa is based on a daily supply of five million litres of hot thermal water of low mineral content with a mean concentration of 40 nCi ^{222}Rn /litre. Furthermore, since 1949 a former goldmine (the Thermal Gallery), with a temperature of up to 41°C, a relative humidity of 95%, and a mean ^{222}Rn -content of 3 nCi/litre air, has been included in the treatment facilities [12]. As almost all the ^{222}Rn dissolved in the thermal water emanates into the indoor and outdoor atmosphere, people living in this region receive an elevated chronic alpha radiation burden from the inhalation of ^{222}Rn and its decay products. Those working either in or near the thermal bathroom areas, or within or near the Thermal Gallery are additionally exposed to higher burdens, fractionally received during the working hours. From



CHRONIC GAMMA BURDEN: ● CONTINUAL, POPULATION IN BADGASTEIN [14], [19], ■ CONTINUAL, POPULATION IN BRAZIL, BUT WITH ABRUPTLY CHANGING RADIATION FIELD [28], ▲ WORKERS, MONAZIT ORE MILL, BRAZIL [28], ▲ WORKERS, NUCLEAR INDUSTRY, USA [29], ▼ WORKERS, AAECRE, AUSTRALIA [30], ♥ WORKERS, UKAEA, UK [31], ♦ WORKERS, RADIATION CENTER, DENMARK [32].

CHRONIC ALPHA BURDEN: □ CONTINUAL, ^{226}Ra BURDEN, LUMINOUS DIAL PAINTERS, UK [33], ◇ CONTINUAL, ^{226}Ra BURDEN, LUMINIZING INDUSTRY, CZECHOSLOVAKIA [34], ◊ CONTINUAL, ^{232}Th BURDEN, THOROTRAST PATIENTS, AUSTRIA [35], ○ WORKERS, THERMAL GALLERY, BADGASTEIN [14], [19], △ URANIUM MINERS, USA [36].

ACUTE LOW LET IRRADIATION: ★ PATIENTS, IN VIVO, PARTIAL BODY, USA [37], † IN VIVO, ACCIDENTAL, WHOLE BODY, ^{60}Co -GAMMA, USA [27], * IN VITRO, ^{60}Co -GAMMA, USSR [9], + IN VITRO, X-RAYS, USA [10], × IN VITRO, X-RAY, UK [11], • IN VITRO, ^{60}Co -GAMMA, USA [38], ◦ IN VITRO, X-RAY, USA [39], ⊙ IN VITRO, X-RAY, USA [40], ▼ IN VITRO, X-RAY, UK [26], ▲ IN VITRO, ^{60}Co -GAMMA, UK [26].

FIG.4. Dose-response curve of chromosome breaks combined from data of various authors.

each person blood was sampled on a number of occasions from the cubital vein and put into culture using routine techniques. Early harvests were scored and from 30590 cells all deviations from the normal noted. Preliminary results of the cytogenetic investigations have been published [13, 14].

The dose calculation was based on extended measurements of all the components of environmental radioactivity carried out for many years. Details on these measurements have been given elsewhere [15, 16]. The external gamma dose as well as significant mean values for ^{222}Rn and its short-lived daughters in the air were repeatedly measured in every single living, sleeping and working room of each of the test persons as well as outdoors. On the basis of these measurements, and on information collected from each person individually on the amount of time spent at the working place, at home, in the open air and elsewhere, we calculated the 'alpha blood' dose from inhalation of the radioactive nuclides as well as the 'gamma blood' dose from external irradiation. The method of calculating the organ dose from inhalation of ^{222}Rn and its decay products is given in detail in previous publications [17, 18], and the formula for the gamma blood dose is discussed in Ref.[14].

In Fig.3 the sum of chromosome breaks⁴ is plotted against the sum of alpha and gamma blood dose accumulated over one year before blood sampling. The aberrations have been corrected to an age of 50 years. The standard errors have been calculated according to the assumption of a Poisson distribution. This dose-effect curve rises sharply up to an annual burden of 200 to 300 mrad yearly mainly from continual gamma irradiation.⁵ In the region where the gamma burden is of the same order of magnitude as the additional alpha burden, occupationally received within 2 to 4 h a day, the curve flattens into a plateau. This represents those persons entering the Thermal Gallery who received an additional alpha burden for 2 or 4 h daily which exceeded the gamma dose by a factor of up to 10.

3. FITTING OF OWN RESULTS WITH DATA OF OTHER AUTHORS

In Fig.4 we have plotted the sum of chromosome breaks from material available in the literature against the 'blood dose'. We regarded the 'sum of breaks' as the most adequate criterion of injuries due to ionizing irradiation, as chromosome breaks are assumed to be correlated with DNA breaks caused by energy deposition within the cell. Furthermore, in our investigation dose dependence was best shown by the sum of chromosome breaks [19]. This is in accordance with what was found by others for the total number of aberrations [9]. For a comparison of the results of various authors we have to compare aberration rates according to radiation burden times ranging from continuous to acute irradiation.

⁴ Deletions counted as one break; dicentrics, minutes and rings as two breaks.

⁵ The ratio of alpha to gamma dose ranged between 0.007 and 0.1.

Here the problem arises of deciding on a suitable accumulation time for chronic irradiation. We considered one year to be a reasonable mean value for the persistence of cells with chromosome aberrations. It is known that a number of aberrations disappear within several weeks after irradiation [20], although lymphocytes carrying chromosome breaks have been known to survive up to 20 years with a mean half life of 1.5 to 5 years [21]. For chronic irradiation, a kind of equilibrium between appearance and disappearance of aberrations will occur. We found that chromosome aberrations in the blood lymphocytes induced by high-level cranial irradiation of children with acute lymphatic leukaemia had largely disappeared one year after treatment [22].

To estimate the accumulated annual dose for the blood we had to recalculate the data of the other authors. Where the radiation burden in WLM had been given, we transferred this into annual alpha blood doses using our investigations on the dose distribution pattern caused by inhalation of ^{222}Rn and its short-lived decay products [18]. To convert the ^{226}Ra body burdens, we used the factor from Spiers and Burch [23] who calculated that 1 nCi of ^{226}Ra whole-body burden causes a dose rate of 0.8 mrad yearly for soft tissues. The blood doses for Thorotrast patients could be only roughly estimated. We used values of Grillmeier [24] and Parr [25] on the distribution of ^{232}Th in the body and on the excretion of ^{220}Rn through the blood. The dosimetry of Thorotrast is, however, complicated by the unequal distribution of the body load. Where the external gamma field was given we transferred the exposure dose (R/y) into blood dose (rad/y) using factors discussed in Ref. [14].

In Fig. 4 are also plotted the chromosome aberration yields found by several authors at high doses of low LET irradiation in in-vitro cultures of human blood lymphocytes. In spite of differences in radiation quality, dose rate and culture techniques, the dose effect curve above 100 rads fits well the dose square kinetics, namely a straight line in the log-log scale (e.g. Ref. [26]).

Our comparison of the effects after 'in-vivo' and 'in-vitro' irradiation is justified by experimental investigations of numerous authors (e.g. Ref. [27]), who found essentially the same chromosome aberration rates for both kinds of irradiation. The comparison of chromosome aberrations due to acute dose with those due to chronic dose can be justified by theoretical considerations, namely that at low doses the effects are mainly due to one hit events which are dose-rate independent, irrespective of LET.

4. CONCLUSIONS

For the reasons given above, the influence of time, kind, quality and method of irradiation is not considered, and the dose effect curve as presented in Fig. 4 is considered to be a reasonable representation of the available data. If this is

so, we must conclude the following: at very low doses (Range A) the biological effects rise sharply with dose. At higher doses (Range B) the effects are either not or only weakly dose-dependent appearing as a plateau or flat curve. Only at doses above about 30 rads (Range C) are the well-known dose kinetics followed, best described by the two component theory [2, 3]. Therefore it will never be possible to extrapolate the linear parts of Range C to Range A or vice versa. To combine the high-dose response curve with that at lowest doses it would be necessary to assume that the hitherto accepted hypotheses do not apply for Range B, where the effects do not respond to dose.

5. DISCUSSION

In drawing overall conclusions regarding the shape of the dose-effect curve at low doses, considerations of differences in dose rates, LET and in-vitro or in-vivo irradiation were omitted. From our detailed investigations, however, differences in aberration frequencies with dose rates do exist. We found the highest effects for continually irradiated persons. This contradicts what is usually found for low LET irradiation at high dose. But our findings agree with the higher aberration rates obtained for continuous irradiation caused by the deposition of radionuclides in the body. Although these burdens derive from high LET alpha irradiation we tend to attribute the higher aberration yields rather to a dose-rate effect than to the effect of LET, as our workers and uranium miners fractionally irradiated with alpha rays show fewer aberrations at comparable doses. Furthermore, the good agreement of values derived from comparable doses of gamma and alpha burden support the tenet that the influence of LET is negligible at low radiation burdens.

As previously mentioned, the doses calculated for chronic irradiation depend on the accumulation time which we took to be one year. A longer or shorter period would shift values in Range A either to the right or left. However, only an accumulation time of 30 to 50 years, which is certainly incorrect, would allow a direct extrapolation of high to low doses.

The shape of the dose-effect curve in Fig.4 could be caused by cellular repair mechanisms. Repair enzyme activity is most probably regulated in a manner analogous to other, inducible enzymes. If the damage is low it will be repaired by the more or less constant pool of available enzymes (Range A). A moderate elevation of damage would trigger an additional amount of enzyme production resulting in a higher repair capacity thereby changing the dose-damage relation (Range B). At higher levels of damage, however, the inducible repair capacity is exhausted, and the degree of effect again rises with dose.

The observation of a sharp rise in the initial dose-effect curve followed by a plateau or at least a much flatter shape is also described in other dose-dependent biological phenomena.

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DISCUSSION

G.W. DOLPHIN: You are trying to establish a dose-effect relationship for in-vivo irradiation of lymphocytes. I think it is impossible to calculate the dose to the lymphocytes in the radiation exposures you describe.

J. POHL-RÜLING: We have been working for more than ten years on the problem of the dose distribution pattern in the human body, especially that from inhaled radon and its daughters, as you can see from Refs [17] and [18]. As the dose to the lymphocytes we took the blood dose (including the dose that the blood receives while passing through the lungs) which we have been able to estimate quite accurately. The fact that the lymphocytes are mainly pooled in other organs (liver, spleen, etc.) does not have much effect on the dose estimation. The doses to these organs differ from the blood dose by a factor of three at the most, and this would not affect our conclusions.

L.G. LITTLEFIELD: Estimating dose in persons contaminated with alpha emitters is extremely difficult. We have also been interested in this problem in relation to americium-241. In a recent in-vitro dose-response system, in which human lymphocytes were exposed to alpha radiation from ^{241}Am , we found an RBE for ^{241}Am alpha radiation of 40–60 at doses of 1 to 5 rads. Thus, there may be a problem in plotting your gamma and alpha data together in terms of dose in rads.

J. POHL-RÜLING: The RBE of alpha irradiation at low doses certainly is still unknown for most biological effects, so we had no choice but to give the physical dose (in rads). The dose in rem is given only for radiation protection purposes. However, the slope of the dose effect curve appearing in our Fig.4 is mainly due to low-LET irradiation (black signs) and would not be modified even if the dose of the white signs were multiplied by a factor of 50.

DOSE-EFFECT RELATIONSHIPS FOR MALIGNANCY IN CELLS WITH DIFFERENT GENETIC CHARACTERISTICS

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Abstract

DOSE-EFFECT RELATIONSHIPS FOR MALIGNANCY IN CELLS WITH DIFFERENT GENETIC CHARACTERISTICS.

By combining the proposals that malignancy behaves as a recessive genetic character, that a somatic mutation is an important step in the development of cancer, and that radiation-induced DNA double-strand breaks are the critical lesions which may lead to cell death, mutation and chromosomal aberrations, considerations can be made and equations derived for the incidence of malignancy in cells having different genotypes. Equations are derived for diploid carrier cells and tetraploid carrier cells, and are compared with data in literature on cell transformation. It is shown that some differences in experimental results could be due to the different genetic character of the cells used. The theoretical considerations are extended to the population which is considered to be constituted of 'carriers' and 'non-carriers' of the recessive malignant genotype. The possible influence of radiation on 'non-carriers' is discussed as are the implications of the presence of two groups within the population for the estimation of risk to low doses of radiation.

INTRODUCTION

The use of cell transformation techniques makes it possible to study the biology of radiation-induced malignancy at a cellular level. One of the most important aspects of this study concerns the definition of the shape of the dose relationship for cell transformation. Information on the shape of the dose relationship will undoubtedly influence further considerations on the derivation of risk estimates for cancer induction from low doses of radiation for the population.

Dose relationships for cell transformation by ionizing radiation have been published for two different cell lines. Borek and Hall [1] have published the response for primary hamster embryo cells; Terzaghi and Little [2] have published the response for an established C3H mouse embryo cell culture. The two dose relationships differ in both shape and frequency of transformation.

In this study, we wish to show that a combination of the proposals that malignancy behaves as a recessive character and that a somatic mutation can

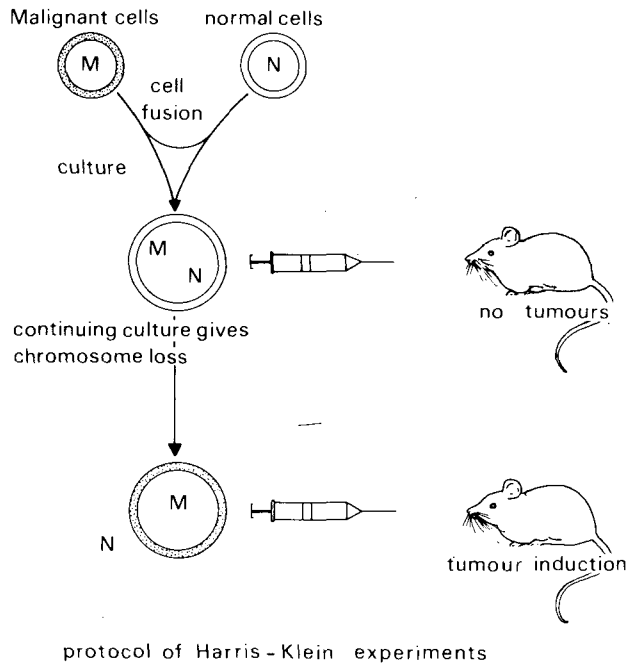


FIG.1. Schematic representation of the cell fusion experiments which led to the conclusion that the factor that governed malignancy behaved as a recessive genetic character.

cause malignancy with the molecular theory of radiation action can lead to a logical explanation of the different dose relationships which is due solely to the different genetic characteristics of the two cell lines.

MALIGNANCY AS A RECESSIVE GENOTYPE

In a series of experiments Harris, Klein and their colleagues [3–10] have demonstrated that the factor which governs malignancy is carried by a chromosome and behaves as a recessive genetic character, i.e. the expression of malignancy is suppressed by the presence of a normal homologous chromosome.

The protocol for these elegant experiments is presented in Fig.1. Malignant mouse cells, derived from several different tumours, were fused with non-malignant mouse cells; different clones of fused hybrid cells were cultured and inoculated into genetically compatible newborn, X-irradiated mice. It was found that the fused hybrids were non-malignant initially, but that following continued propagation of the hybrid cells *in vitro* the malignant phenotype often

reappeared. The reappearance of the malignant phenotype was accompanied by chromosome loss. The results of the experiments have been interpreted as indicating that the factor which governs malignancy can be carried by a cell which is non-malignant until the normal homologous chromosome, which suppresses the malignant genotype, is lost from the cell. These workers have also found that one normal chromosome can suppress the action of more than one homologous recessive malignant chromosome.

These results have all been obtained using mouse cells, but Stanbridge [11] has recently repeated the experiments using human cells with the same results. It would appear that the recessive nature of the malignant genotype is a general feature of mammalian eukaryotic cells.

It is clear that in a cell which carries the malignant genotype (a carrier cell), a mutation in the normal suppressive chromosome can remove the suppressive action and make the cell malignant; it is equally clear that another mutation may just as easily remove the malignant genotype itself.

The results of Harris and Klein and Stanbridge provide a logical basis for the results of Ames and co-workers [12, 13] who have found a strong correlation between the mutagenic and carcinogenic action of a large number of different chemical and physical agents, and also for the proposal that a somatic mutation is an important step in the initiation of malignancy.

THE MOLECULAR THEORY OF RADIATION ACTION

The molecular theory of radiation action has been developed from the basic assumption that a radiation-induced DNA double-strand break is the most critical lesion which may lead to either cell death, chromosomal aberrations and somatic mutations. We have assumed that the mean number (N) of DNA double-strand breaks per cell induced by a dose of radiation (D) is given by

$$N = \alpha D + \beta D^2 \quad (1)$$

where α represents the average number per unit dose of double-strand breaks that are induced in single radiation events, and β represents the average number per unit dose squared of double-strand breaks that arise from the combination of two unrepaired single-strand breaks. The coefficients α and β contain parameters to take into account the repair of single- and double-strand breaks.

The linear-quadratic dose relationship for the induction of double-strand breaks in cells has been confirmed by the work of Dugle and co-workers [14].

In the development of the theory (see Ref. [15] for review) we have derived the following equations:

$$\text{Survival } S = \exp - p(\alpha D + \beta D^2) \quad (2)$$

$$\text{Aberration Yield } Y = k(\alpha D + \beta D^2) \quad (3)$$

Mutation induction per surviving cell

$$M = 1 - \exp[-q(\alpha D + \beta D^2)] \quad (4)$$

$$\cong q(\alpha D + \beta D^2) \quad (5)$$

Combining Eqs (2) and (3), and (2) and (5) gives

$$\ln S = -(p/k)Y \text{ and } \ln S = -(p/q)M$$

These predicted correlations between survival and chromosomal aberration yield, and between survival and mutation induction, have been found in practice [15–23] and indicate that the basic molecular lesion which leads to each biological end point is of the same type.

By combining the proposals of Harris and Klein on the recessive genetic nature of malignancy with the molecular theory of radiation action equations can be derived to describe the dose relationship for cell transformation.

The dose relationship for a diploid carrier cell

In a non-malignant diploid carrier cell an aberration or mutation in the normal homologous chromosome will remove the suppressive factor of this chromosome and transform the cell into a malignant state. However, as is shown in Fig.2, a second aberration or mutation in the carrier chromosome may eliminate the malignant genotype itself, and the initially transformed cell would be unable to express the malignant genotype and would be 'reverted'.

Mathematically the transformation equation must therefore consist of two parts, one to describe the probability of transformation by inactivation of the suppressive factor, $1 - \exp - q(\alpha D + \beta D^2)$, one to describe the probability of the cell not being reverted by inactivation of the malignant factor, $\exp - s(\alpha D + \beta D^2)$. Thus the equation for the dose relationship for cell transformation in a diploid carrier cell is given by

$$T = \{1 - \exp[-q(\alpha D + \beta D^2)]\} \exp[-s(\alpha D + \beta D^2)] \quad (6)$$

In Fig.2 this equation has been used to fit data published by Borek and Hall [1] on the cell transformation of primary hamster embryo cells. In fitting

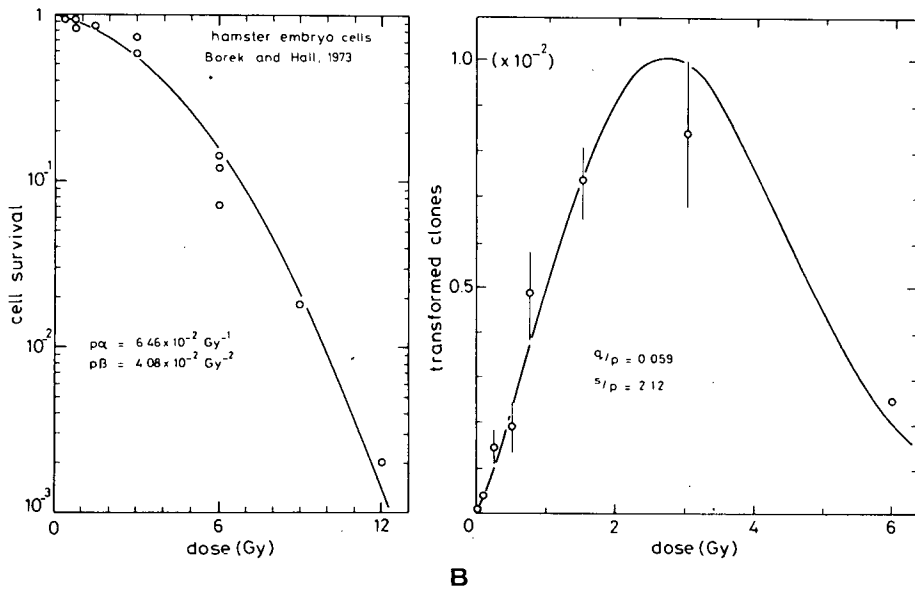
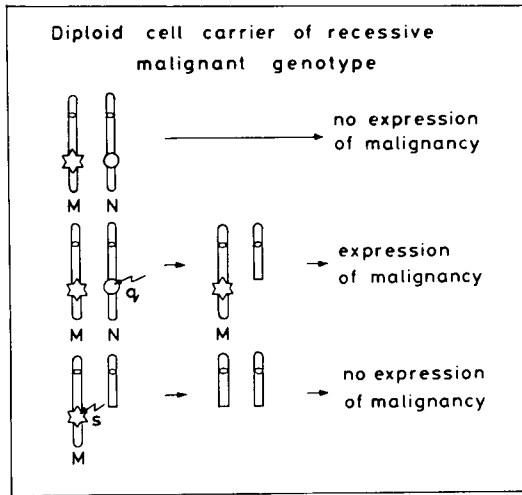


FIG.2. A. Schematic representation of how radiation can induce and suppress malignancy in a diploid carrier cell.

B. The fitting of Equations (2) and (6) to the data of Borek and Hall [1] for survival and transformation of hamster embryo cells.

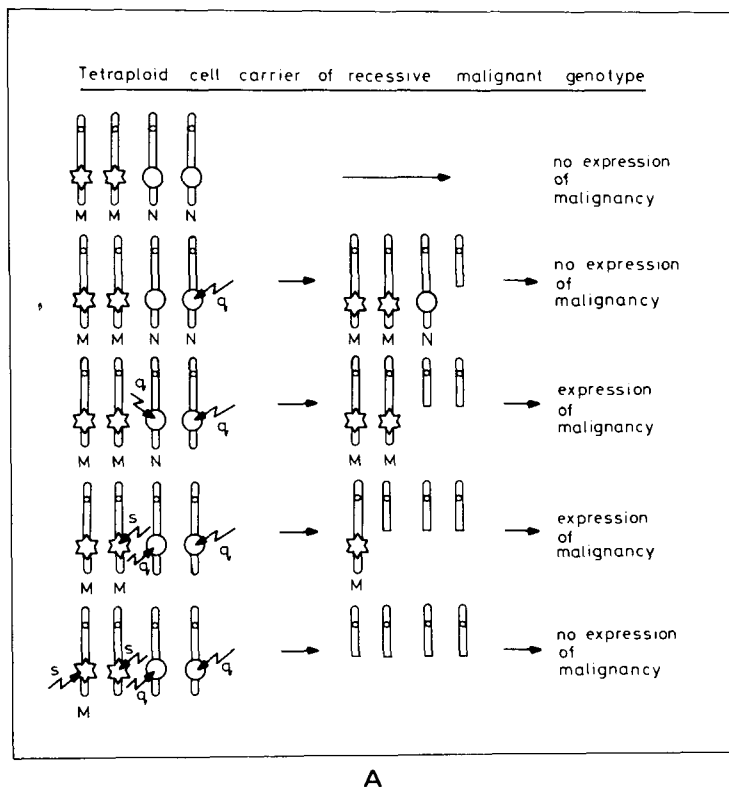
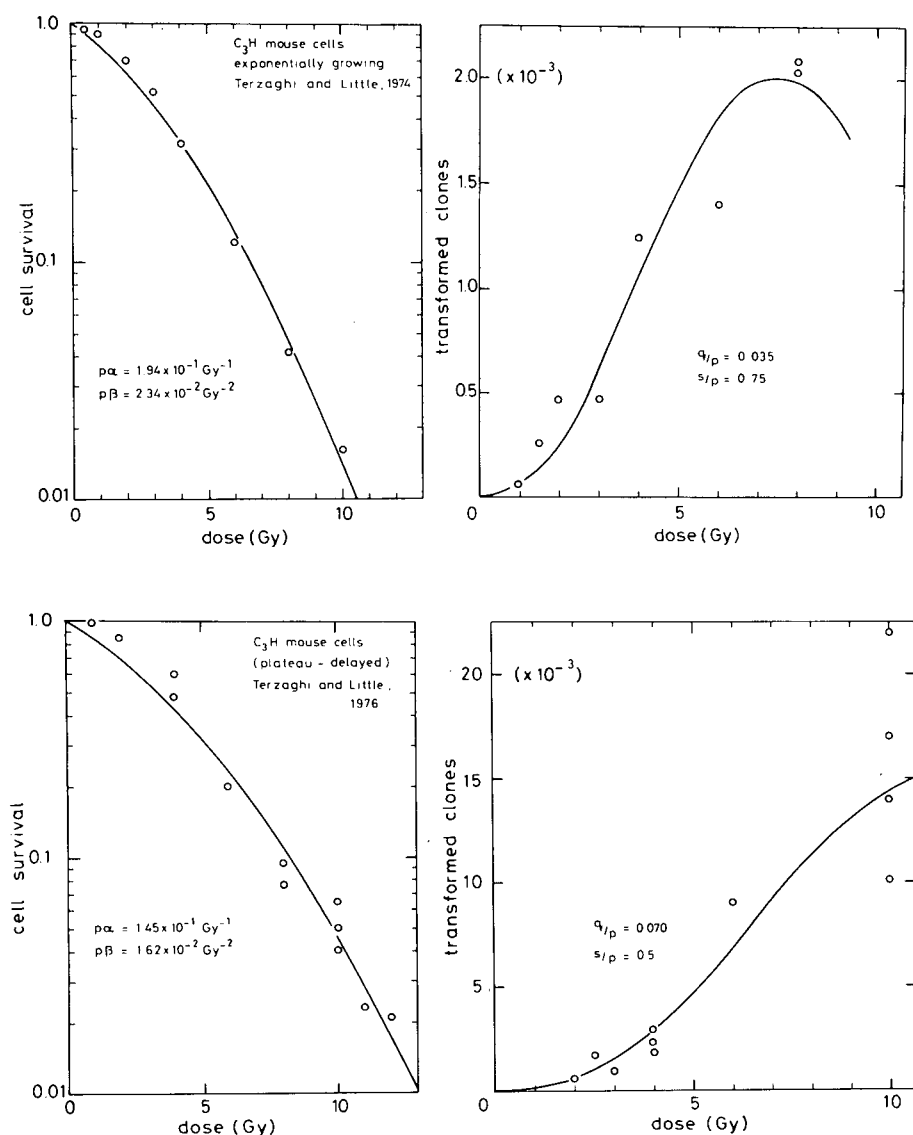


FIG.3. A. Schematic representation of how radiation can induce and suppress malignancy in a tetraploid carrier cell.

B. The fitting of Equations (2) and (7) to the data of Terzaghi and Little [2, 24] for survival and transformation of C3H mouse cells.

this equation the survival of the cells was first analysed using Eq.(2), and the same dose kinetics, in α and β , were used for the transformation curve which is matched by varying the values of q and s only.

One important feature of Eq.(6) which arises from the results of Harris and Klein, independent of the radiation theory, is that although the cell transformation per surviving cell has been determined the curve must pass through a peak and decrease at higher doses. This decrease at higher doses is not related to the preferential killing of transformed cells, but is a direct result of the proposals of Harris and Klein and the fact that radiation can remove the malignant genotype from the cell and 'revert' it.



B

The dose relationship for a tetraploid carrier cell

In a non-malignant tetraploid carrier cell two copies of the malignant genotype and two copies of the suppressive homologous chromosome will be present. As Harris and Klein have shown that one suppressive chromosome can prevent the expression of malignancy, even when more than one malignant genotype is present, the tetraploid cell will only become malignant when both suppressive chromosomes have been mutated and will only be 'reverted' when both malignant genotypes have been mutated (see Fig.3).

In this case the transformation equation will consist of the probability that both suppressive chromosomes are mutated, $(1 - \exp - q(\alpha D + \alpha D^2))^2$, and the probability that both malignant genotypes are not mutated, i.e. that the cell is not 'reverted', $(1 - [1 - \exp - s(\alpha D + \beta D^2)])^2$. Thus the equation for the dose relationship for cell transformation in a tetraploid carrier cell is given by

$$T_t = \{1 - \exp[-q(\alpha D + \beta D^2)]\}^2 \{1 - (1 - \exp[-s(\alpha D + \beta D^2)])^2\} \quad (7)$$

In Fig.3 this equation has been used to fit data published by Terzaghi and Little [2, 24] on the transformation of C3H(10T- $\frac{1}{2}$) clone 8 mouse cells in exponential growth and in plateau phase with a 3-hour delay in plating. This cell line is stable and has been characterized as being hypertetraploid [25]. The survival of the cells has been analysed using Eq.(2), and the relevant dose kinetics in α and β have been used to fit the transformation data by varying q and s .

DISCUSSION

Cell transformation

The experimental results of Borek and Hall [1] and Terzaghi and Little indicate quite clearly certain important differences. The shape of the cell transformation dose response relationship is different, and the cell transformation frequency for the C3H cells is an order of magnitude smaller than that found for the Hamster embryo cells. The analysis presented here demonstrates that according to the proposals of Harris and Klein, and independent of any radiation theory, it is to be expected that the dose relationships are radically different because of the different genetic make-up of the cells. What the combination of the molecular theory of radiation action with the proposals of Harris and Klein gives is a compatible mathematical description of all the different data including the survival of the cells. It also automatically explains the difference in cell transformation frequency for the two cell types even though the coefficients in $p\alpha$, $p\beta$, q/p and s/p are quite similar. The differences are simply due to the fact that one cell type is diploid and the other is tetraploid.

As a consequence of this analysis it would appear that although the C3H cell line may have considerable advantages for the investigation of cell transformation, because it is a stable cultured line with a low spontaneous incidence of cell transformation, its tetraploid nature precludes its use for the determination of the shape of the dose-response relationship to radiation of any sort to indicate the dose relationship for animals and man. It is worth noting that the low spontaneous incidence of cell transformation results most probably from the tetraploid nature of the cell.

It is also important to note that the analysis automatically, and logically, provides an explanation for the decrease in cell transformation per surviving cell at high doses. This effect would also explain the results of Proukakis [26] and Lindop [27], who found a decreasing incidence of malignancy at high doses although the same number of surviving cells were injected into a host animal.

In Fig.4 we show an analysis of the Terzaghi and Little data [2, 24] made in a different way because we feel that, although this analysis is not completely valid, there is some evidence which supports it, and it reveals some interesting extra information. In making the analysis we have assumed that the survival of the exponentially growing cells is similar to that of plateau-phase cells plated immediately after irradiation; this has been indicated by Terzaghi and Little [24]. The difference between the two survival curves shown in Fig.3 is ascribed to the repair of double-strand breaks during the 3-hour delay, and leads to the relationship between the two survival curves indicated by the log-log plot in Fig.4 [28]; the repair factor which is obtained is in agreement with that found by Terzaghi and Little [2] for plateau-phase cells at 1200 rads. On this basis we have fitted the two curves for cell transformation by using the same $p\alpha$ and $p\beta$ coefficients and varying q and s . In the fit shown q_2 (3-hour delay) is approximately twice q_1 (exp) but s_2 decreases such that s_2/s_1 is the same factor as has been found for the two survival curves. This indicates that the two types of mutations are different in nature, and that repair increases the probability that the suppressive factor is removed to expose the malignant genome, and that repair reduces the probability that the malignant genotype itself is lost. It is interesting to note, in addition, that the probability that the malignant genotype is mutated (s) is an order of magnitude greater than the probability that the suppressive factor is mutated (q) in all three analyses.

The human population

The human population is probably made up of a mixture of carriers and non-carriers, that is, people who carry in their diploid cells one chromosome which carries the malignant genotype that is normally suppressed by its homologue (the carriers), and a group who do not carry the malignant genotype at all (non-carriers). The occurrence of cancers in families is the most obvious evidence for

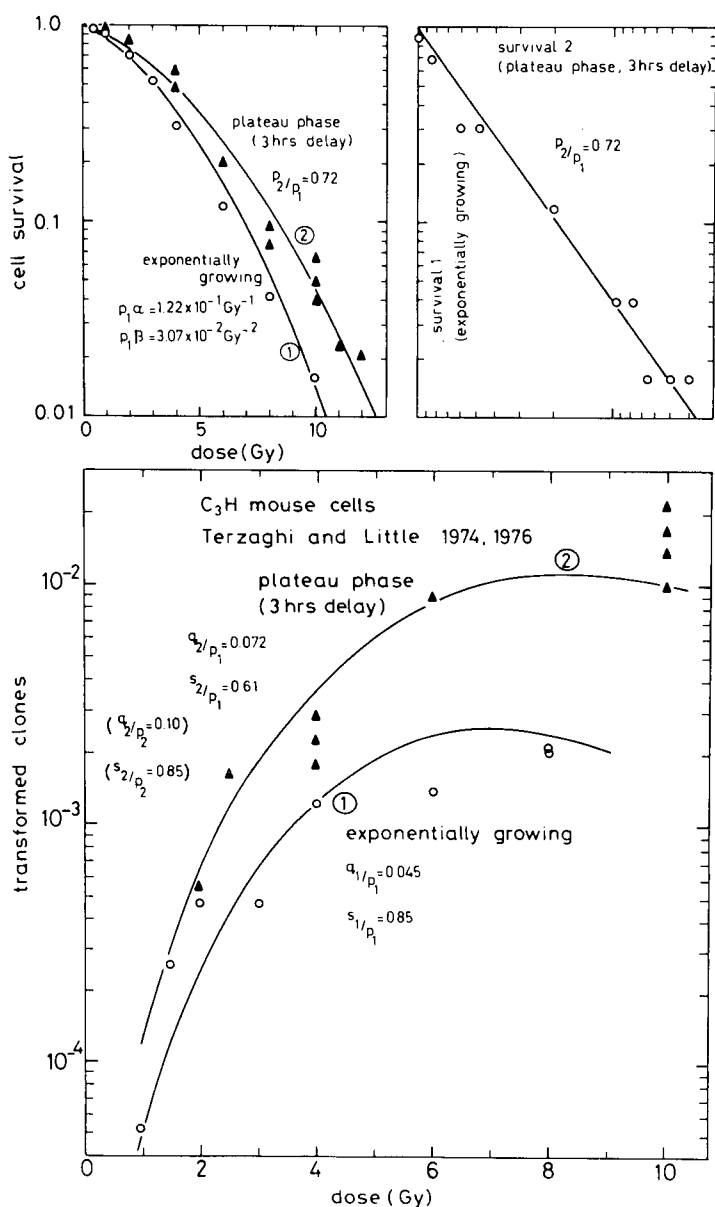


FIG. 4. A different approach to the fitting of the data of Terzaghi and Little [2, 24]. In this case the difference in survival has been assumed to be dependent only on the repair of DNA double-strand breaks, and this repair is assumed also to be responsible for the difference in the incidence of cell transformation.

the identification of a carrier group, and the idea that some people have a 'pre-disposition' to cancer is becoming more generally accepted. There is also evidence from animal studies that some strains, usually showing a high spontaneous incidence of cancer, are also sensitive to the induction of cancer by radiation [29]. In the human population the carrier group will form a sensitive group for the induction of cancer by radiation and other mutagenic/carcinogenic agents.

On the other hand, the question of whether radiation can ever induce cancer in the non-carrying group remains completely open. It all depends whether radiation can convert a normal chromosome, via mutation, into one carrying the malignant genotype, and this probably depends on whether the factor which governs malignancy is inherent in the cells' genetic information or whether it has to be incorporated as extra DNA via, for example, a virus. This remains a mute point, but in our opinion it is one that deserves more attention and which might lead to information on the role of viruses and other carcinogenic agents in cancer. In any case the non-carrier group will form either a low incidence, or zero incidence, group with respect to the induction of cancer by radiation.

In this respect there are some interesting results from cell transformation experiments which demonstrated that in some cells it was apparently impossible to induce cell transformation by radiation [29–32]. These results could be explained if the cells were non-carriers and radiation is unable to convert a non-carrier into a carrier cell.

It is possible to derive the equation for the percentage incidence (P) of a specific cancer in the human population from Eq.(6) as

$$P = 100E (1 - \exp[-nTS]) \quad (8)$$

where E is the fraction of carriers in the total population

n is the number of cells per person which can be transformed to give the specific cancer by radiation

T is Eq.(6) and represents the probability or chance for cell transformation in a diploid/carrier form

and S is Eq.(2) and represents the chance that the transformed cell survives to express its malignant character

We have demonstrated the fitting of Eq.(8) to data for the induction of myeloid leukaemia in mice by different types of radiation [33] in a previous publication [15].

The suggested existence of a sensitive carrier group within the total population is of importance in radiological protection. If such a group exists, then in assessing the risk to low doses of radiation where use has been made of the incidence of cancer in general groups of the population [34, 35] the risk to the sensitive group has probably been seriously underestimated. The question is whether or not some special risk levels should be derived for this sensitive

group, and if so, how can such levels be derived and how can the sensitive group be identified.

In considering the possible existence of a sensitive and non-sensitive group we wondered if this might provide an explanation for some of the results of the recently published Hanford analysis [36] where the workers who had died from cancer had accumulated on the average a higher radiation dose than other workers who had died from other causes, even though a third group, still living, had received even higher radiation doses. Perhaps the workers dying from cancer form the sensitive group selected retrospectively.

CONCLUSION

The most important conclusion resulting from this analysis is that the shape of the dose-response relationship for cell transformation and the incidence level are both critically dependent on the genotype of the cell, and that the results for the tetraploid C3H cell line cannot be directly extrapolated to the case for man. In cell transformation studies it is important to define the genotype of the cell with respect to its potentially malignant character.

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DISCUSSION

P. FISCHER: The hypothesis on which this study was planned, namely that malignancy is a recessive character carried on one gene, is based on the somatic cell hybridization studies of Harris and Klein, who found that in a hybrid between a malignant and a non-malignant cell malignancy was suppressed, but would reappear after chromosome loss had occurred. It has not been possible so far to substantiate this theory by demonstrating that malignant suppression is associated with the loss of one specific chromosome. It would therefore seem that malignancy has a polygenic origin.

K.H. CHADWICK: I appreciate that the proposals of Harris and Klein are not completely established, and that in fact Harris himself is not satisfied that all is right; however, we developed this model in 1974 on the basis of their reports and, as you will appreciate, it provides a very satisfactory explanation of the radiation cell transformation data. In fact, irrespective of the radiation theory used, the proposals of Harris and Klein lead to a logical explanation of several problems.

Y. NISHIWAKI: In your equations for survival, aberration yield and mutation induction per surviving cell, you assume the same values of α and β . Are there any cases where different values of α and β have to be assumed for these different types of effect?

K.H. CHADWICK: In the induction of the DNA double-strand breaks, which is described by α and β , the same values for the end points are assumed but, if for instance the survival is determined by the unrepaired double-strand breaks and mutations by the misrepaired double-strand breaks, then under different repair conditions we can expect a shift in the proportion of breaks contributing to the two end effects. This can be taken into account by a shift in p , q and s (although this is not strictly mathematically correct — a repair factor should be introduced). The relationship of α to β does not change.

DOSE-INCIDENCE RELATIONSHIPS OF RETICULUM CELL SARCOMA IN MICE

Observations and hypotheses at the cellular level

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Abstract

DOSE-INCIDENCE RELATIONSHIPS OF RETICULUM CELL SARCOMA IN MICE: OBSERVATIONS AND HYPOTHESES AT THE CELLULAR LEVEL.

The final incidence of reticulum-cell sarcoma (RCS) in normal BCF₁ mice is about 50% and is not changed by the administration of single acute whole-body doses of X-rays of up to 400 rads; higher doses produce a decrease of incidence down to a few per cent. Doses of 400 rads given to bone marrow before its injection into heavily irradiated syngenic hosts significantly increase the incidence and rate of appearance of RCS, in comparison with non-irradiated marrow: this observation suggests that marrow contains cells susceptible to radiation-induced neoplastic transformation. Recently, observations have been extended to include an endogenous repopulating system where the marrow is irradiated in situ with moderate doses by a limb-shielding technique, and allowed to repopulate the rest of the body which is heavily irradiated. In these animals the incidence of RCS as a function of dose is described by a biphasic curve having a peak at about 500 rads. The initial ascending portion of this curve is in agreement with previous data obtained in the exogenous system, and the final descending portion at doses in excess of 500 rads parallels the trend observed in whole-body irradiated animals. For a given dose level to the moderately irradiated marrow, shielding of both legs results in a higher frequency of RCS than shielding of one leg only. These observations suggest the existence of a positive correlation between the mass of irradiated marrow (i.e. the number of susceptible cells) and the frequency of tumour appearance. It is possible, under a few reasonable simplifying assumptions and making use of independent estimates of the number of cells at risk and of their radiation response, to derive some relationship between the probability of cellular transformation and radiation dose: it is also possible to show that this relationship holds for various experiments and experimental conditions.

INTRODUCTION

The dose-effect relationships for systemic neoplasms of the lymphoreticular tissues in mice show different shapes, depending on the tumour type and the mouse strain [1]. In particular, relatively undifferentiated tumours classified as

reticulum cell sarcomas (RCS) and non-thymic generalized lymphomas are characterized by a decreasing frequency as a function of increasing radiation doses [1, 2]; this negative trend has been found in different strains and with various dose ranges. RCS is therefore one of the rare tumour types that appears to be inhibited rather than enhanced by radiation in experimental mice.

The aim of this report is to show that, under particular conditions of partial body irradiation, reticular tumours can be induced by irradiation of the bone marrow. Furthermore, by collecting all the data now available from our laboratory on whole-body and partial-body irradiation, and on the syngenic chimaera system, it is possible to derive some qualitative relationship between the probability of *in vivo* transformation to potentially neoplastic cells and the radiation dose, in the range 0–800 rads. Only a few reasonable and simplifying assumptions, and two independent estimates of the numbers of cells at risk and of their radiation response, are necessary to formulate such a relationship, which holds for various experiments and experimental conditions.

ANIMALS AND EXPERIMENTAL PROCEDURES

All the animals used in the experiments discussed in this paper were hybrid (C57Bl/Cne × C3H/Cne)F₁ male mice (BCF₁), bred and maintained in the animal house of the Laboratory for Experimental Animals at the Centre for Nuclear Studies of CNEN, Casaccia. They were housed three-to-five to a cage and kept under standardized conventional conditions of environmental and hygienic care. Full randomization procedures were always used to assign the mice to various experiments and experimental groups. The animals were usually three months old at the time of treatment; when radiation doses were in the lethal range, only survivors at 60 days were included in the life-span observations, and post-treatment survival times of all groups were normalized accordingly. The techniques of pathology at spontaneous death, of histologic examinations and of statistical treatments of the final results have been described in detail previously [3, 4], as well as the standard methods of haemopoietic cell transplantation and titration in terms of surviving colony-forming units. All irradiations were performed with 250-kVcp X-rays, HVL = 1.5 mm Cu, at dose rates of 35 to 135 rads/min approximately. Only mice that had to be immobilized for partial-body irradiation were anaesthetized with ip sodium pentobarbital just before irradiation; to account for the radiobiological protective effects of general anaesthesia, the lethal dose under this condition was 1000 rads, compared with 900 rads given to unrestrained unanaesthetized recipients of haemopoietic cells. The procedure for partial-body and limb-shielding exposure was as follows: the anaesthetized mice were firmly secured to a pressed-wood board, and either one or both the hind legs were covered by appropriate lead shields kept in place for

only part of the time required to irradiate the rest of the body with the lethal dose. During a brief interruption of irradiation the shields were removed so that for the rest of the treatment time the part(s) previously shielded were fully exposed to the beam. Appropriate variations of the shielding time allowed irradiation of the bone marrow of the limb(s) with doses between 0 and 800 rads. The long-term survival of these animals was therefore dependent on the autologous haemo- and lymphopoietic repopulation starting from surviving totipotent stem cells of the limb marrow [5].

SUMMARY OF EXPERIMENTAL RESULTS

The results of three experimental series are summarized here to support the final discussion; some of these results have been partially or fully reported previously, and only the limb-shielding experiments will be described in some detail.

Whole-body irradiation

Several experiments were completed between 1967 and 1975, the animals being simply irradiated with single graded doses of X-rays and observed until spontaneous death [6]. The average final incidence of RCS in these experiments is plotted as a function of dose in Fig.1. The point at 900 rads is the average frequency of RCS in syngenic radiation chimaeras repopulated by exogenous transplantation of between 5 and 5500 unirradiated bone marrow CFUs [3, 4]. Little variations are observed for doses up to about 400 rads, and only higher doses sharply decrease the final incidence. On the semilog plot the final portion of this curve approaches an exponential fall with an inverse slope of the same magnitude as the D_0 of bone marrow CFUs estimated *in vivo* in the same strain of animals (unpublished data). In comparison with RF females, for which similar dose-incidence data are available at moderate doses [1], the BCF₁ males are even less sensitive to radiation, in terms of RCS frequency changes.

Irradiated bone marrow transplantation

The original data have been fully described previously [3, 4]. In essence, irradiation *in vivo* of bone marrow cells immediately before quantitative transplantation into 900-rads-irradiated isogenic recipients increases the incidence and rates of RCS, when compared with transplantation of unirradiated marrow. The increase in final incidence is statistically significant at 400 rads, and the age-specific death rate is increased also at the 200-rads dose level. The average frequency of RCS from these experiments is plotted in Fig.1.

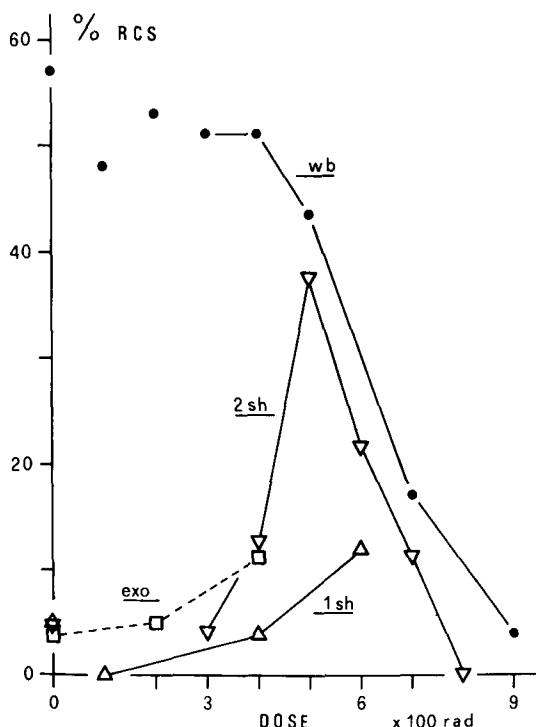


FIG.1. Final incidence of RCS as a function of dose under three different experimental conditions: (a) whole-body irradiation (wb), (b) shielding of one (1 sh) or two (2 sh) hind legs, (c) bone marrow transplantation in syngenic chimaeras (exo).

Whole-body irradiation with limb shielding

By this technique the animals are repopulated by autologous surviving stem cells; the general pattern of survival and RCS frequency after zero-dose to the shielded limbs has already been reported [5], and shows that this model system is essentially superimposable on the syngenic chimaera model after transplantation of unirradiated marrow (Fig.1). After moderate and high radiation doses to the shielded marrow, the number of animals surviving the short-term radiation syndromes is small and variable, as was expected on general radiobiological grounds, but long-term survival is comparable at all marrow doses and for both one-leg and two-leg shielding conditions. Some two-leg-shielded animals were recovered for life-span observations after marrow doses as high as 800 rads. The frequency of RCS in these animals has been plotted in panel A of Fig.2. A simple χ^2 test showed a significant heterogeneity of frequencies with respect to the marrow doses, and the general shapes of the two curves are therefore the expression of

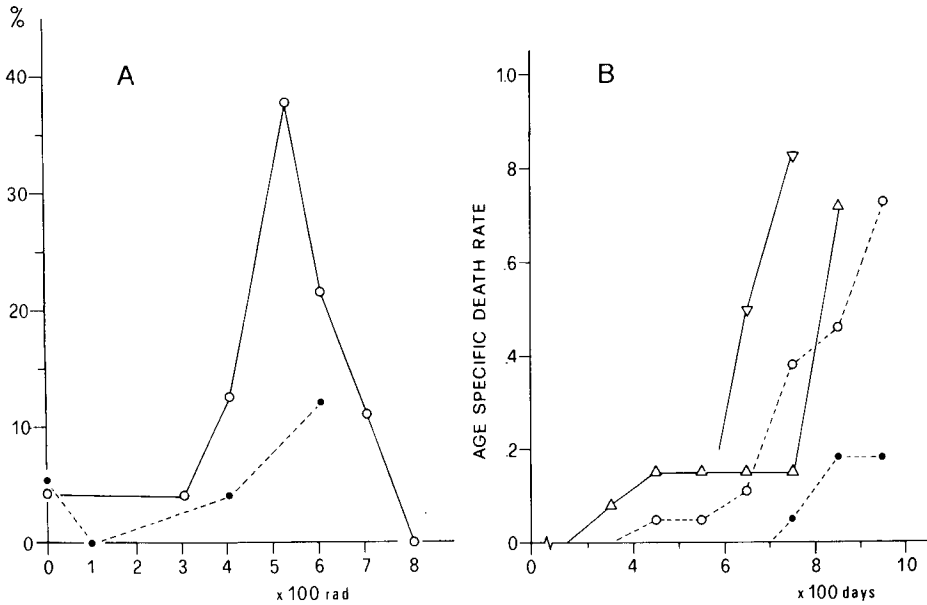


FIG. 2. A. Dose incidence curve for reticulum cell sarcoma in the one-leg-(●) and two-leg-(○)-irradiated animals.

B. Cumulative age-specific death rate for reticulum cell sarcoma as a function of post-treatment survival time in two-leg-irradiated series; (▽) single dose of 600; (△) 500 rads; (●) 0 rad to the shielded marrow; (○) unirradiated control.

well-defined trend of tumour development as a function of radiation dose to the marrow. Particularly in the two-leg-irradiated animals, a clear biphasic dose-effect relationship shows a peak incidence at about 500 rads, followed by a sharply decreasing trend. Further evidence of radiation induction of RCS is given by the age-specific death rate analysis, plotted in panel B of the same figure; this type of analysis clearly demonstrates that lymphoreticular tumours in marrow-irradiated animals are expressed with much shorter latency times; only the data for 500 and 600 rads to both hind legs are reported and compared with the completely shielded marrow, which plots at the extreme right hand side of the panel. Actually, the rate of RCS in these mice approaches the high rates which are typical of control unirradiated animals of the same strain [3, 4].

DISCUSSION

As mentioned in the introduction, the inverse relationship of the RCS frequency with dose in the whole-body irradiated animal is confirmed by our

data and extended to a wide range of doses. The prevalence of cell killing over concomitant transformation events at all dose levels might be taken as the simplest hypothesis to account for the unusual behaviour of RCS [7]. This hypothesis is, however, insufficient, unless some additional assumptions are made either about differences in sensitivity between normal and transformed cells, or about differences in the shape of the dose-effect relationship for cell killing and cell transformation. None of these differences have been experimentally verified at the cellular level *in vivo*. It was only pointed out, as stated above, that in BCF₁ male mice the final portion of the dose-frequency curve for RCS approached the slope of the survival curve of haemopoietic stem cells in the same mouse strain, and this remark would point to similarities rather than differences in cell inactivation kinetics. In essence, therefore, observations from whole-body irradiation of mice would lead to the conclusion that RCS is a tumour type which is not induced by radiation.

In contrast, it was shown in our laboratory [4] that the transplantation of bone marrow immediately after whole-body irradiation of donors with 200 or 400 rads into heavily irradiated isogenic hosts significantly increases the incidence and rates of RCS in comparison with the transfer of unirradiated marrow. This observation strongly indicates that the excess tumours seen under these conditions might be correlated with the radiation dose to the marrow, and that some normal marrow cell types are susceptible to radiation-induced transformation to potentially neoplastic cells. Similar data were obtained in other experiments [8] where heavily irradiated recipients were repopulated by cells from different haemopoietic tissues of donors pre-treated with highly leukaemogenic irradiation regimes. Excess tumours with increased rate of appearance were seen, for instance, in animals receiving spleen cells or marrow cells from donors irradiated with fractionated doses (4×150 rads) at 4–8 weeks of age. Although it was not possible to show directly in our mouse strain that the extra tumours were of donor origin because of the lack of suitable cellular markers, these additional observations pointed to the validity of the syngenic chimaera model, and to its capacity for detecting qualitatively similar trends in various experimental situations. However, a severe limitation of this model system was the narrow range of radiation doses that could be given to the cells to be transplanted; in most cases, and even at moderate doses, the surviving fraction of stem cells would be too small to ensure short- and long-term survival of the recipients and to observe the late expression of tumours.

The recent application of partial-body irradiation, although less precise than the exogenous system in the absolute quantitation of initial cell numbers, provided more direct evidence regarding some critical problems on RCS induction. Firstly, the data of Fig.2 leave no doubts that reticular tumours are, in fact, induced by doses of radiation higher than 400 rads, which could not be tested by the exogenous system. Secondly, the same data show that the tumours seen under

this particular condition must find their origin in cells of the unshielded marrow, transformed by radiation and later expressed in the same animal. Thirdly, the dose-incidence variations under partial-body irradiation clearly show the same pattern as seen for many radiation-induced tumour types, including other systemic lymphoreticular neoplasms such as thymic lymphoma and myeloid leukaemia [1], with an ascending portion at low and moderate doses up to a peak, followed by a decreasing trend at higher doses. The close parallelism of this last portion to that of whole-body irradiation points to similarity of mechanisms. Finally, the comparison between one-leg- and two-leg-irradiated animals indicates a close correlation between the frequency of tumours and the mass of irradiated marrow, hence the number of cells at risk, at all the dose levels where data are available for such a comparison.

In the discussion to follow an attempt will be made to derive a qualitative relationship between the probability of *in vivo* transformation to potentially neoplastic cells and the radiation dose, making use of the following assumptions:

- (1) the cells capable of neoplastic transformation are the bone marrow stem cells;
- (2) the average number of stem cells per whole mouse and in different sections of the haemopoietic system is known;
- (3) the kinetics of stem cell killing *in vivo* is also known, and there is no differential sensitivity between normal and transformed cells to radiation-induced lethality;
- (4) one surviving transformed cell is sufficient to express one tumour.

There is no direct experimental evidence to support the first hypothesis; the data summarized here and discussed at length previously [3, 4, 8] suggest, however, that this hypothesis is reasonable, in view of the long latency times of RCS expression which by itself requires that transformed cells must have long-lasting stemness potential. The number of total haemopoietic stem cells in a mouse of our strain has been estimated to be on the average about 5×10^5 by two independent methods, one based on radiobiological evidence [4], and the other on direct titration of spleen colony-forming cells in the femur and on the distribution of erithropoietic marrow in the various body districts [4, 9]. The latter information provides also estimates concerning the numbers of stem cells contained in the unshielded limbs under partial-body irradiation. As for the third hypothesis, direct experimental data show that stem cell killing *in vivo* is an exponential function of the dose with D_0 estimated as 75 rads (unpublished data). Finally, the last hypothesis cannot be proved; however, it does not seem to be critical for this analysis, because if any initial transformed-cell multiplicity conditions are required for the long-term expression of the tumours, these conditions might be expected to remain on the average constant in all the experimental models discussed here. This expectation may not hold true if the above conditions are strongly dependent on radiation dose, but no data are as yet available on this last problem.

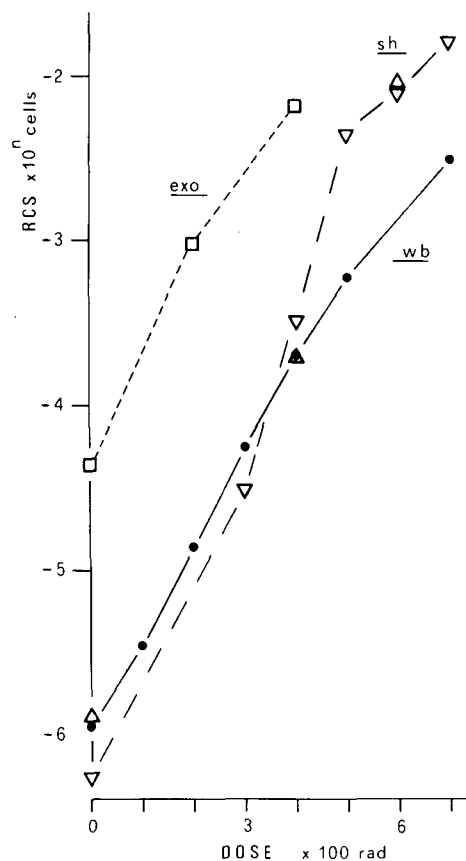


FIG.3. Numbers of observed RCS per surviving stem cell as a function of radiation dose to the marrow in whole-body-irradiated (wb), hind-leg-shielded (sh) and bone-marrow-transplanted (exo) mice.

The average number of surviving stem cells per mouse was calculated at each dose level for the three experimental conditions (whole-body, one-leg and two-leg irradiation); the number of reticular tumours seen in the animals irradiated under the three conditions was then divided by the estimated number of surviving cells, and the resulting ratio has been plotted in Fig.3 as a function of radiation dose to the marrow. The three points already calculated for the exogenous system [4] are reported in the graph for a direct comparison with the endogenous systems. Under the assumptions discussed above, Fig.3 represents the probability of transformation to potentially neoplastic clonogenic cells of normal surviving bone marrow cells as a function of radiation dose.

Some unproven and simplifying assumptions have been made to derive this relationship, and therefore a full discussion does not seem to be warranted at the present stage of the experiments. However, some comments are pertinent here. Firstly, the consistency of the shapes of the curves should be pointed out, under widely different conditions of both initial radiation exposure and biological environment of late tumour expression. The relationship between dose and cell transformation probability is close to an exponential function of the dose over a wide range of both doses and initial cell survival. Secondly, this sort of analysis proved to be successful in reconciling the large discrepancy of final frequency data between whole-body irradiation and the other conditions for doses lower than about 500 rads, as seen in Fig.1. A common mechanism of induction may then be postulated at the cellular level, and the differences in tumour final incidence might be explained by the combined effects of stem cell multiplicity, stem cell killing and cell transformation probability as a function of radiation dose.

In conclusion, the data summarized here, and the preliminary analysis carried out under some simple hypotheses, would indicate that an approach is now possible to investigate tumour induction events at the cellular level *in vivo*, provided that some particular experimental conditions are identified, whereby the numbers of cells at risk and some of their radiobiological properties are initially known or can be estimated. This information can be used as further controlled experimental variables, in addition to the radiation dose and to the final frequency of tumour per animal. Further research is needed to fully validate some of the assumptions and to refine quantitatively the relationship of Fig.3; the degree of generalization of this analysis to other mouse strains and tumour types remains also to be established.

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DOSE-RESPONSE CURVES FROM INCOMPLETE DATA

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Abstract

DOSE-RESPONSE CURVES FROM INCOMPLETE DATA.

Frequently many different responses occur in populations (animal or human) exposed to ionizing radiation. Interest usually focuses on one particular endpoint (e.g. radiation-induced leukaemia). To obtain a dose-response curve, the exposed population is first divided into sub-groups whose members received the same radiation dose. To estimate the response, the fraction of subjects in each sub-group that showed the particular response of interest is determined. These fractions are plotted against dose to give the dose-response curve. Such curves for different populations are then compared, and many investigators hope to get insight into the underlying mechanism of radiation-induced malignancies from the shape of these curves. This procedure of plotting the fractions versus the radiation dose is not the correct way to estimate the time distribution for a particular response at the different dose levels. Other observed responses competed for the individuals in the exposed population and therefore prevented manifestation of the complete information on the response-time distribution for one specific response. Such data are called incomplete in the statistical literature. A procedure is described which uses the by now classical Kaplan-Meier estimator, to establish dose-response curves from incomplete data under the assumption that the different observed responses are statistically independent. It is demonstrated that there is insufficient information in the observed survival functions to estimate the time distribution for one particular response if the assumption of independence is dropped. In addition, it is not possible to determine from the data (i.e. type of response and when it occurred) whether or not the different response-time distributions are independent. However, it is possible to give sharp bounds between which the response has to lie. This implies that for incomplete data, only a 'dose-response band' can be established if independence of the competing responses cannot be assumed. For incomplete data the shape of the dose-response curve is therefore undecidable in some situations. Examples are given using actual data to illustrate the estimation procedures.

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1. INTRODUCTION

The 'shape' of dose-response curves for different types of radiation (e.g. high or low LET) has been the topic of innumerable discussions at many meetings. This symposium is no exception. Linear, quadratic and even 'supra-linear' curves are being considered, and mechanisms which might produce such dose-response curves are being debated. A closer look at a dose-response curve shows that it 'consists' of several estimated points corresponding to the doses to which the subjects (animals or men) were exposed. Usually dose is plotted on the abscissa and incidence on the ordinate. It is customary to estimate incidence by the fraction (number of subjects showing a particular response)/(total number of subjects exposed) for each dose level. A simple example shows how problematic this procedure is. Assume that five animals were exposed to a certain dose D_1 from external gamma radiation. In the first experiment three leukaemias occur at 3, 5 and 7 units of time after exposure. In the second experiment three leukaemias occur at 2, 4 and 6 units of time. The remaining two animals in the first group died of lung tumours at 4 and 6 units of time. Their counterparts in the second experiment died because they were accidentally dropped by two technicians at 3 and 5 units of time. According to the standard procedure, the incidence is $3/5$ in both cases. But it is clear that the data in the two experiments are quite different for two reasons:

(1) In the first experiment the responses of interest — leukaemias — occurred at different times than in the second experiment. The fraction defined above does not make use of this information. (2) In the second experiment accidents competed with the leukaemias for the lives of the five animals. In the first experiment lung tumours were the competing cause of death. Again the fraction $3/5$ does not attempt to make use of this fact.

Both experiments have one feature in common: the information on leukaemia which the investigators hoped to obtain was in a sense 'incomplete', because the occurrence of the unfortunate accidents and the unavoidable lung tumours 'censored' a part of the knowledge about leukaemia. This is the reason why such data are called incomplete or censored data in the statistical literature. The occurrence of the censoring causes of death represents, therefore, a 'loss' of information. Some authors call these censoring causes 'losses' for this very reason. Subsequently, incidence for such censored data will be redefined using established statistical procedures. The resulting estimates for the incidence will be different from the 'fractional' estimates, and will therefore influence the shape of the dose-response curve. These procedures will be used to analyse data from experiments with beagles receiving injections of ^{226}Ra and ^{228}Th .

2. DOSE-RESPONSE CURVES FROM CENSORED DATA

This section consists of two parts. First, the case will be discussed when the competing causes of death (e.g. accidents in experiment 2 above) and the response of interest (leukaemia in the example given above) are assumed to be statistically independent. In the second part, will be stated what can be estimated if the response of interest and the censoring causes of death are assumed to be statistically dependent.

2.1. The response of interest and the losses are assumed to be statistically independent

An abstract definition of statistical independence will not be given. Instead, this concept will be illustrated with the help of the example given in the introduction. It is clear that the accidental deaths of the two animals in the second experiment did not influence the process of leukaemogenesis in these same animals. Therefore it is safe to assume independence of leukaemias and accidental deaths. The 'best' estimate of the cumulative time distribution for censored data with independent losses was given by Kaplan and Meier [1]. Since this paper is well known and readily available, the detailed mathematical form of the Kaplan-Meier (K-M) estimator will not be given here, but one should note that it considers the times when the response of interest occurred amid the losses. The K-M estimator will therefore differentiate between the two experiments mentioned earlier, but only to the extent that it weighs the times of occurrence 'properly'. It does not use the fact that the losses in the two experiments are of a different type (accidents and lung tumours). The estimate for incidence at a given dose D is defined as the value of the Kaplan-Meier estimator at the longest time for which it is defined. The domain over which it is defined is discussed in Ref.[1]. This definition is meaningful because the K-M procedure estimates the cumulative time distribution for the response of interest, and the values of a cumulative distribution give the proportion of animals that showed the response of interest before or at a time t . The values of the K-M estimators at the different dose-levels for the maximum common time T at which all these estimators are defined, give the incidences for each dose level. It is important to use values at the same time. Otherwise radiation-induced pathogenesis could progress longer at certain doses than at others. A plot of the values of the K-M estimators versus the corresponding doses gives a dose-response curve corrected for independent competing causes of death. The dose-response curves for the data [2] in Tables I and II are shown in Figs 1 and 2. The fractional estimates (\circ) are also shown. The shape of the curves in Figs 1 and 2 obtained with the K-M procedure is very similar to the shape of the dose-response curve for ^{226}Ra in man reported by Evans [3]. This suggests a similar mechanism for osteosarcoma induction [4].

TABLE I. RESPONSE TIMES FOR BONE SARCOMAS IN BEAGLES INJECTED WITH RADIUM-226

Number of			Mean amount injected (μ Ci/kg body weight)	Response times (days) (— Indicates bone sarcoma) ^a																
Level	Dogs	Bone sarcomas		10 481,	9 <u>825,</u>	8 <u>908,</u>	7 <u>968,</u>	6 <u>1015,</u>	5 <u>1091,</u>	4 <u>1220,</u>	3 <u>1288,</u>	22 <u>1288,</u>	1 <u>1380</u>							
5.0	10	9	10.4	13 490,	12 <u>1324,</u>	11 <u>1435,</u>	10 <u>1469,</u>	9 <u>1471,</u>	8 <u>1518,</u>	7 <u>1553,</u>	6 <u>1606,</u>	5 <u>1614,</u>	4 <u>1647,</u>	3 <u>1659,</u>	2 <u>1884,</u>	1 <u>1939</u>				
4.0	13	12	3.21	12 1610,	11 <u>1737,</u>	10 <u>1897,</u>	9 <u>1917,</u>	8 <u>1932,</u>	7 <u>2099,</u>	6 <u>2226,</u>	5 <u>2487,</u>	4 <u>2497,</u>	3 <u>2612,</u>	2 <u>2850,</u>	1 <u>2955</u>					
3.0	12	11	1.07	13 325,	12 2948,	11 <u>3297,</u>	10 <u>3425,</u>	9 3440,	8 <u>3569,</u>	7 <u>3775,</u>	6 3998,	5 <u>4368,</u>	4 4459,	3 4615,	2 <u>4703,</u>	1 4781				
2.0	13	6	0.339	14 557,	13 1273,	12 2399,	11 2577,	10 3142,	9 3254,	8 3267,	7 3432,	6 3914,	5 <u>4107,</u>	4 4438,	3 4903,	2 5320,	1 5495			
1.7	14	1	0.166	22 893,	21 1729,	20 2038,	19 2988,	18 3544,	17 <u>3612,</u>	16 3625,	15 3662,	14 3717,	13 3739,	12 3745,	11 3780,	10 3860				
1.0	22	1	0.062	9 3978,	8 3991	7 4003,	6 4054,	5 4376,	4 4399,	3 5260,	2 5462,	1 5727								
0.5	10	0	0.022	3192,	3676,	3717,	3745,	3822,	3963,	3991,	4141,	4297,	4542							
0.2	10	0	0.0074	3101,	3387,	3448,	3493,	3611,	3662,	3991,	4102,	4190,	4518							
0.0	22	0	0.000	5284	Maximal loss time ^b															

^a The number above the response and loss times indicates the reverse rank. It is given because the K-M estimator in Eq.(1) can conveniently be expressed as a function of the reverse rank.

^b No bone sarcomas occurred at the 0.0 level. Only the maximal loss time is given to keep the table compact. A few beagles are still alive. All loss times can be found in Ref.[2].

2.2. The response of interest and the losses are assumed to be statistically dependent

As in Section 2.1., the concept of statistical dependence is only illustrated with the example given earlier. In the first experiment it is conceivable that leukaemogenesis was influenced by the development of lung tumours. The probability of occurrence for leukaemia depended therefore on tumorigenesis.

In this case the joint survival function $H(l,r)$ for the times to loss (loss time l) and for the time of occurrence of a response of interest (response time r) cannot be estimated. This is shown using an argument given by Birnbaum [5]:

$$H(l,r) = \text{Prob}\{L > l, R > r\} = \int_l^\infty \int_r^\infty f(u,v) du dv \quad (1)$$

When the experiment is terminated we know for each animal that a loss (e.g. accident) or a response (e.g. leukaemia) occurred. In addition we know when the event occurred. For $l < r$ one can write $H(l,r)$ from Eq.(1) in the following fashion:

$$H(l,r) = \text{Prob}\{r < R < L\} + \text{Prob}\{l < L < R\} - \text{Prob}\{l < L < R < r\} \quad (2)$$

The last term in Eq.(2) cannot be estimated from the knowledge of the event (loss or response) and the time when it occurred (L or R) because it would require knowledge of both L and R for one individual. Therefore $H(l,r)$ cannot be estimated. It immediately follows that the cumulative response-time distribution

$$1 - H(l = 0, r)$$

also cannot be estimated. One lacks information on both L and R . What can be estimated if the response and the losses are statistically dependent? The answer was found by Peterson [6], who showed that sharp upper and lower bounds for the cumulative time distribution can be estimated. This means that the incidence, defined in Section 2.1 as the value of the cumulative response-time distribution at time T , has to lie between these bounds. These bounds are far apart if losses occur frequently and they collapse if only the response of interest occurs [6]. Peterson showed furthermore [7] that independence of the response and the losses cannot be proved, if only the type of event (response or loss) and the time of its occurrence are known. The data from Tables I and II are used again to calculate the Peterson bounds, which are shown in Figs 1 and 2. Since these bounds are sometimes far apart (see the figures) the shape of the dose-response curve is not well defined. It is clear that more information is needed to find out what the 'real' incidence is.

TABLE II. RESPONSE TIMES FOR BONE SARCOMAS IN BEAGLES INJECTED WITH THORIUM-228

Number of Level Dogs Bone sarcomas			Mean amount injected (μ Ci/kg body weight)	Response times (days) (—— Indicates bone sarcoma) ^a														
5.0	2	0	2.70	97,	212													
4.0	4	2	0.858	4 <u>645,</u>	3 763,	2 793,	1 <u>833</u>											
3.0	12	12	0.290	12 <u>547,</u>	11 <u>685,</u>	10 <u>801,</u>	9 <u>804,</u>	8 <u>826,</u>	7 <u>859,</u>	6 <u>861,</u>	5 <u>890,</u>	4 <u>971,</u>	3 <u>988,</u>	2 <u>1062,</u>	1 <u>1156</u>			
2.0	13	12	0.0919	13 78,	12 <u>1015,</u>	11 <u>1022,</u>	10 <u>1038,</u>	9 <u>1078,</u>	8 <u>1085,</u>	7 <u>1108,</u>	6 <u>1209,</u>	5 <u>1222,</u>	4 <u>1234,</u>	3 <u>1282,</u>	2 <u>1449,</u>	1 <u>1541</u>		
1.5	13	10	0.0302	13 380,	12 <u>1624,</u>	11 <u>1859,</u>	10 1921,	9 <u>2120,</u>	8 <u>2309,</u>	7 <u>2373,</u>	6 <u>2408,</u>	5 <u>2576,</u>	4 <u>2665,</u>	3 <u>2894,</u>	2 <u>2983,</u>	1 <u>3110</u>		
1.0	12	5	0.0152	12 1263,	11 2546,	10 2886,	9 <u>3172,</u>	8 <u>3217,</u>	7 3273,	6 <u>3420,</u>	5 <u>3538,</u>	4 <u>4034,</u>	3 4142,	2 4570,	1 5298			
0.5	12	2	0.00518	12 1682,	11 1976,	10 2159,	9 3032,	8 3471,	7 3952,	6 4149,	5 4518,	4 <u>4599,</u>	3 4856,	2 <u>4947,</u>	1 5840			
0.2	13	0	0.00171	889,	3350,	3897,	3897,	4217,	4515,	4573,	4720,	4767,	4822,	4826,	4837,	5049		
0.0	13	0	0.00000	171,	1412,	2592,	3072,	4137,	4271,	4549,	4700,	4895,	4963,	5061,	5306,	5510		

^a The number above the response and loss times indicates the reverse rank. It is given because the K-M estimator in Eq.(1) can conveniently be expressed as a function of the reverse rank.

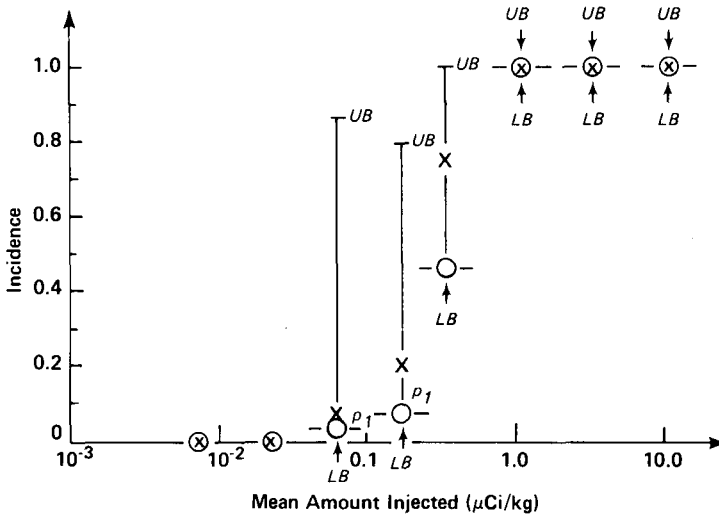


FIG. 1. Dose-response curve for bone sarcomas induced by ^{226}Ra in beagles: X = K-M estimates at 4781 days; O = fractional estimates; * = the coincidence of the two estimates; — = bounds; U(L)B = upper (lower) bounds; arrows indicate the collapse of bounds with estimated points (—X—, —O—) or with each other.

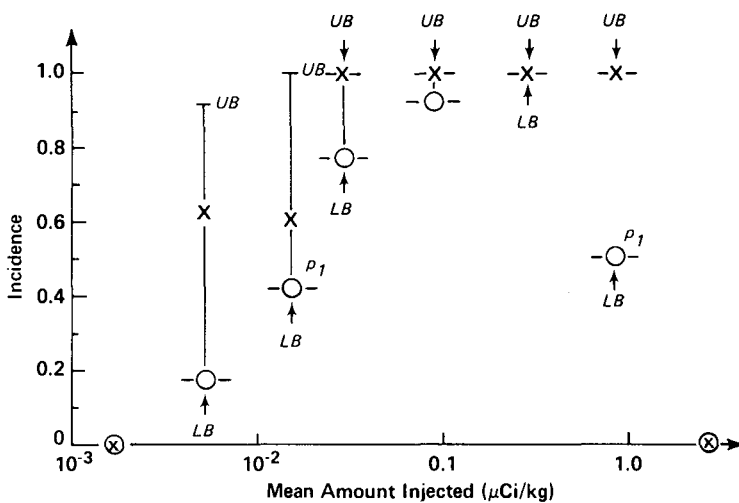


FIG. 2. Dose-response curve for bone sarcomas induced by ^{228}Th in beagles: X = K-M estimates at 5298 days; O = fractional estimates; * = the coincidence of the two estimates; — = bounds; U(L)B = upper (lower) bounds; arrows indicate the collapse of bounds with estimated points (—X—, —O—) or with each other.

3. SUMMARY AND CONCLUSION

Incidence for incomplete data with independent losses has been defined as the value of the K-M estimator at the same time for all dose levels. This produced estimated dose-response curves that are corrected for the occurring losses. Since the K-M estimates for incidence are different from the traditional fractional estimates, the shape of the corresponding dose-response curve will be changed. If the losses are statistically dependent, only sharp upper and lower bounds for the 'true' incidence can be estimated. Since the shape of estimated dose-response curves influences the extrapolation to low doses, a re-analysis of important data (e.g. leukaemias in atomic bomb survivors) seems necessary. All the estimates described earlier are the 'best' possible estimates (see e.g. Ref. [6]) if only cause and time of death are known for all subjects in a population at risk.

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THE QUADRATIC LOW-LET DOSE-EFFECT RELATION FOR LIFE SHORTENING IN MAMMALS

Implications for the assessment of the low-dose hazard to human populations*

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Abstract

THE QUADRATIC LOW-LET DOSE-EFFECT RELATION FOR LIFE SHORTENING IN MAMMALS: IMPLICATIONS FOR THE ASSESSMENT OF THE LOW-DOSE HAZARD TO HUMAN POPULATIONS.

Data on the relation of lifespan shortening to daily dose are analysed for 15 species of mammals from the orders Carnivora, Artiodactyla and Rodentia given daily gamma-ray exposure for the duration of adult life. The analysis is in terms of the relation of mean radiation-specific death rate (estimated as difference of reciprocals of survival times for exposed and control groups) to daily dose. With one exception, all species conform to a generic death rate-dose rate relation, with death rate increasing as the square of dose rate up to a breakpoint, above which death rate increases as the first power of dose rate. The log-log plot of the y co-ordinate (death rate) against the x co-ordinate (dose rate) of the breakpoints yields a linear relation, indicating that the large species variation in sensitivity to chronic radiation is due to a single parameter. A two-parameter mathematical model for chromosome damage accounts for the two-branched relation, and for the dependence on the square of the daily dose. The model yields a hyperbolic relation of logarithm of death rate of continuously irradiated cells to logarithm of dose rate that is asymptotic to a line of slope 2 (square of dose rate) at low dose rate and to a line of slope 1 (first power) at high dose rates. It is concluded that most of the species variance in sensitivity to continuous exposure is due to species differences in a parameter identified as an accumulation time for chromosome damage leading to breaks. It is suggested that this time constant is related to the inter-mitotic interval in haematopoietic stem cell populations.

INTRODUCTION

This paper examines the problem of arriving at a valid estimate of the life-shortening efficacy of ionizing radiations for human populations given continuous low-level exposure. The approach we employ is to determine the

* Work supported by the United States Department of Energy.

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TABLE I. SPECIES USED IN THE PRESENT STUDY. LABORATORY MOUSE STRAINS AND HYBRIDS ARE NOT INCLUDED. THE BODY WEIGHTS AND LIFE EXPECTATIONS FOR THE RODENTS ARE UNPUBLISHED DATA OF SACHER AND STAFFELDT. DATA FOR THE BEAGLE DOG ARE FROM NORRIS, TYLER, SACHER [10] AND PERSONAL COMMUNICATION FROM W. P. NORRIS. THE BODY WEIGHTS ARE AVERAGES OF MALE AND FEMALE WEIGHTS. TOTAL NUMBER OF EXPOSED RODENTS IN LAST COLUMN (SEXES COMBINED)

TAXONOMIC NAME	COMMON NAME	FAMILY	BODY WT G	LIFE EXPECT. DAYS	TOTAL SAMPLE N
<i>Tamias striatus</i>	Chipmunk	Sciuridae	100	~1500	125
<i>Chinchilla laniger</i>	Chinchilla	Chinchillidae	440	~2500	66
<i>Sigmodon hispidus</i>	Cotton rat	Cricetidae	130	510	149
<i>Mus musculus</i> (wild type)	House mouse	Muridae	17	610	192
<i>Praomys natalensis</i> (two series)	Multimammate mouse	Muridae	48	630	391
<i>Rattus rattus</i>	Black rat	Muridae	186	~730	162
<i>Oryzomys palustris</i>	Rice rat	Cricetidae	50	730	47
<i>Peromyscus californicus parasiticus</i>	California mouse (northern)	Cricetidae	60	1070	74
<i>Peromyscus californicus insignis</i>	California mouse (southern)	Cricetidae	60	1300	193
<i>Peromyscus floridanus</i>	Florida mouse	Cricetidae	36	1740	31
<i>Peromyscus gossypinus</i>	Cotton mouse	Cricetidae	31	1830	56
<i>Peromyscus leucopus</i>	White-footed mouse	Cricetidae	23	1420	394
<i>Reithrodontomys humulis</i>	Harvest mouse	Cricetidae	9	710	28
<i>Canis familiaris</i>	Beagle dog (domestic)	Canidae	11 000	4200	--
<i>Capra hircus</i>	Goat (domestic)	Bovidae	65 000	~4400	--
<i>Cavia porcellus</i>	Guinea pig	Caviidae	750	1250	--

functional dependence of life shortening on daily dose for sample groups of animals exposed to ^{60}Co gamma rays over a wide range of daily doses for the duration of adult life (D.o.L.). This procedure has two advantages over the procedure in which animals are given single doses early in life and followed for their lifetimes.

The first advantage, abundantly confirmed in the data given below, is that the D.o.L. method yields an unequivocal dose-dependence function, which is reproduced with great fidelity in replicate trials. The single-dose procedure, for reasons not yet understood, yields differing dose-dependence relations between experiments [1, 2, 3, 4].

The second advantage is that the D.o.L. method is highly efficient and cost-effective, and can yield an accurate dose-dependence function while using a total sample of 100 to 200 animals (Table I), no larger than the number needed for one dose point in a single-dose experiment. As a consequence, we have been able to investigate the life shortening functions for more than a dozen species of mammals by the D.o.L. method. The data can be accounted for by a mathematical model that postulates that cellular radiation injury is due to lethal chromosome rearrangements. The insights gained from the data and the model provide orientation for the further research needed to estimate the human radiation hazard coefficients.

MATERIALS AND METHODS

The new experimental data presented here were obtained from 12 species of rodents, one of which is represented by 2 subspecies (entries 1-13 in Table I). Ten species are mouse-like rodents belonging to the suborder Myomorpha, of which 7 are North American species belonging to the family Cricetidae, and 3 are Old World species in the family Muridae. The chipmunk, which belongs to the suborder of squirrel-like rodents, and the chinchilla, which belongs to the ancient group of South American rodents, are very distantly related to each other and to the myomorph rodents [5]. These 12 species range in body weight from 9 to 440 grams, and in lifespan from less than 2 years to about 8 years (Table I).

The 11 myomorph rodent populations (Table I, lines 3-13) were bred at Argonne National Laboratory from founder stocks that were in most cases trapped in the wild, and in the remaining cases were only a few generations removed from the wild [6, 7]. A random outcross mating system was employed to preserve the outbred wild-type genome. The chipmunks were live-trapped as juveniles on the Argonne site. The chinchillas were ranch-bred and transferred to Argonne National Laboratory as young adults.

The myomorph rodents and the chipmunks were kept in plastic shoebox mouse or rat cages on pine chip bedding, and fed pelleted laboratory rodent chow. The chinchillas were kept in large rat cages on pine chip bedding and fed chinchilla chow. The animal rooms and the gamma irradiation room were kept at nominal 23°C and 40% humidity, and illumination was by fluorescent lighting on a 12:12 light-dark cycle.

In addition to the new data for wild rodents, published information on the survival of 8 inbred and hybrid laboratory mouse genotypes [8, 9] is reanalyzed here by the same methods used for the wild-type rodents. The laboratory mice were exposed in the same radiation facility, and housed, fed and maintained in the same way as the wild rodents.

TABLE II. COORDINATES OF BREAKPOINTS, ACCUMULATION TIMES (γ^{-1}), AND DOSE-EFFECT COEFFICIENTS (λ) FOR ALL SPECIES AND GENOTYPES DISCUSSED HERE. BREAKPOINT COORDINATES ESTIMATED BY LEAST SQUARES, AS DESCRIBED IN MATERIALS AND METHODS. SEXES COMBINED UNLESS OTHERWISE NOTED

	$Y \equiv \gamma$ (DAYS) ⁻¹	X R/DAY	γ^{-1} DAYS	$\lambda \times 10^6$ $R^{-1} \times 10^6$
C57L/J (L)	0.0273	109.0	36.6	251
A/J (A)	0.0193	67.0	51.8	288
C57L x AF ₁ (LAF ₁)	0.0196	85.7	51.0	229
C3Hf/J	0.0348	119.7	28.8	289
BALB/cJ (C)	0.0219	71.6	45.7	306
C57BL/6J (B6)	0.0168	68.6	59.5	245
C57BL6 x BALB/cF ₁ (B6CF ₁)	0.0136	60.2	73.6	225
C57BL6 x BALB/cF ₂ (B6CF ₂)	0.0196	75.6	51.0	259
Chipmunk	0.0153	75.1	65.2	204
Chinchilla	≤ 0.0023	≤ 5	≥ 435	400
Cotton rat	0.0243	92.7	41.1	262
House mouse	0.0194	92.0	51.5	211
Multimammate mouse (two series)	0.0246	94.1	40.6	262
Black rat	≥ 0.033	≥ 125	≤ 30.0	≥ 280
Rice rat	0.0398	67.9	25.1	586
California mouse (northern)	0.0087	23.6	115.3	367
California mouse (southern)	0.0317	91.0	31.5	348
Florida mouse	≥ 0.0458	≥ 128.9	≤ 21.8	≥ 355
Cotton mouse	0.0160	73.9	62.5	216
White-footed mouse	0.0340	95.6	29.4	355
Harvest mouse	0.0235	70.1	42.5	335
Beagle dog	0.0380	40.0	26.3	950
Domestic goat (female)	0.0077	22.5	130.0	342
Domestic goat (male)	≤ 0.0025	≤ 7	≥ 400	330
Guinea pig	0.0047	9.5	212.8	495

In addition to these species, previously published data for four other species, dog [10], guinea pig [11, 12], goat [13] and laboratory rat [14], are examined. The original sources should be consulted for the procedures used in these studies.

Radiation exposures were delivered in a gamma-ray exposure room measuring 7.5 x 7.5 m [9]. The experimental groups lived in the room from entry until death. A ⁶⁰Co source in the center of the room was raised nightly and

the animal cages were positioned on racks around the source so that the animals would receive preassigned dose rates. The strength of the source decreased about 1 per cent per month due to radioactive decay, so the exposure time was increased at monthly intervals to compensate. The daily exposure times increased from about 8 to 12 hours over the life of the source.

The daily doses for our interspecies rodent series were 12, 24, 32, 43, 56, 74, 97 and 125 R/day. Some species did not get the full set of treatments, and one species, the chinchilla, was given an additional treatment of 5 R/day because of its extraordinary radiosensitivity. The inbred and hybrid laboratory mice received these treatments, and were also exposed at 220 R/day. Some genotypes were exposed at several higher daily doses. The dog, guinea pig and goat exposures were done under different experimental conditions with different treatment levels. The total number of animals of each species or genotype is given in Table II. The daily doses for the two Argonne rodent series are based on air doses in Roentgens measured at the centers of the cages.

RESULTS AND ANALYSIS

All data sets are displayed and analyzed in terms of the relation of logarithm of mean radiation-specific death rate, as ordinate, to logarithm of daily dose, in R/day, as abscissa. Mean radiation specific death rate, ρ , is defined as

$$\rho = \frac{1}{t_r^*} - \frac{1}{t_o^*}$$

where t_r^* and t_o^* are mean after-survival times from the beginning of exposure for irradiated and control samples respectively [10]. The data for the 8 laboratory mouse genotypes are displayed in Figure 1, and for the 13 wild-type rodent populations in Figures 2 and 3.

All laboratory inbred and hybrid mice show a common pattern of response, with death rate increasing as the second power of daily dose, then bending over, at about 75 to 100 R/day, to a trend with slope of approximately unity. The eight data sets are fitted with a two-branch function with the slopes of the lower and upper branches fixed at 2 and 1, respectively. Curve fitting is done by finding the x, y coordinates of the breakpoint that minimize the squared deviations of the death rate values from the bent line.

The descriptive model for the estimation is

$$\left. \begin{aligned} L_2: y_2 &= a_2 + 2x_2; N_2 \\ L_1: y_1 &= a_1 + x_1; N_1 \quad (N = N_1 + N_2 - 1) \end{aligned} \right\} 1'$$

where a_2 and a_1 are estimable constants, (x_2, y_2) and (x_1, y_1) are the observed coordinates of log dose rate and log death rate, $N_2 = N - i$ is the number of ordered dose rate values included in L_2 , and $N_1 = i + 1$ ($i = 0, 1, 2, \dots, N$) is the remaining number of points together with the highest dose rate point of the N_2 set. These N_1 points comprise the line L_1 . The constants a_2 and

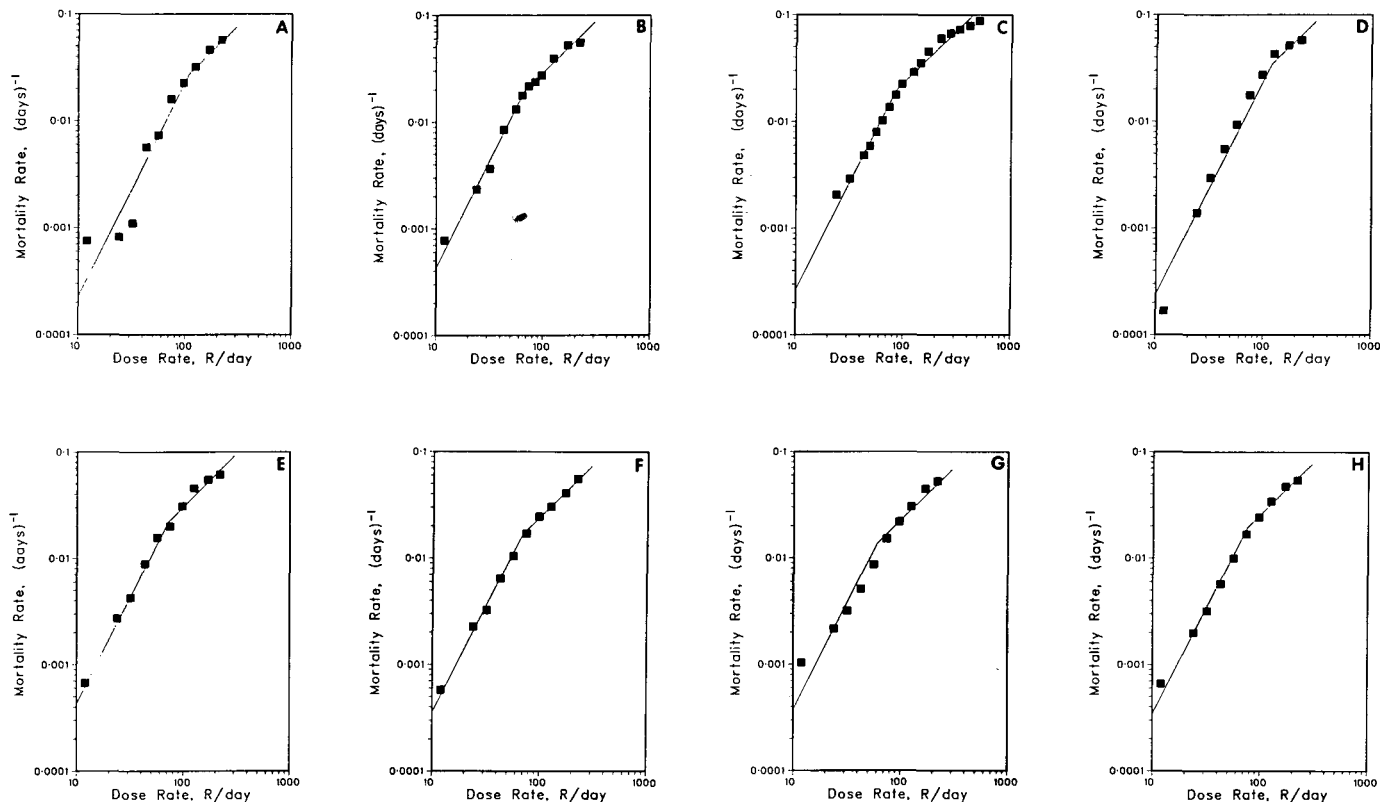


FIG.1. Death rate-dose rate relations for eight inbred and hybrid genotypes of laboratory mice. Two-branched lines fitted by least squares. The genotypes shown (with abbreviations in parentheses) are: (A) C57L/J (L); (B) A/J (A); (C) C57L \times AF₁ (LAF₁); (D) C3Hf/J; (E) BALB/cJ (C); (F) C57BL/6J (B6); (G) C57BL6 \times BALB/cF₁ (B6CF₁); (H) C57BL6 \times BALB/cF₂ (B6CF₂). Data of Grahn [8].

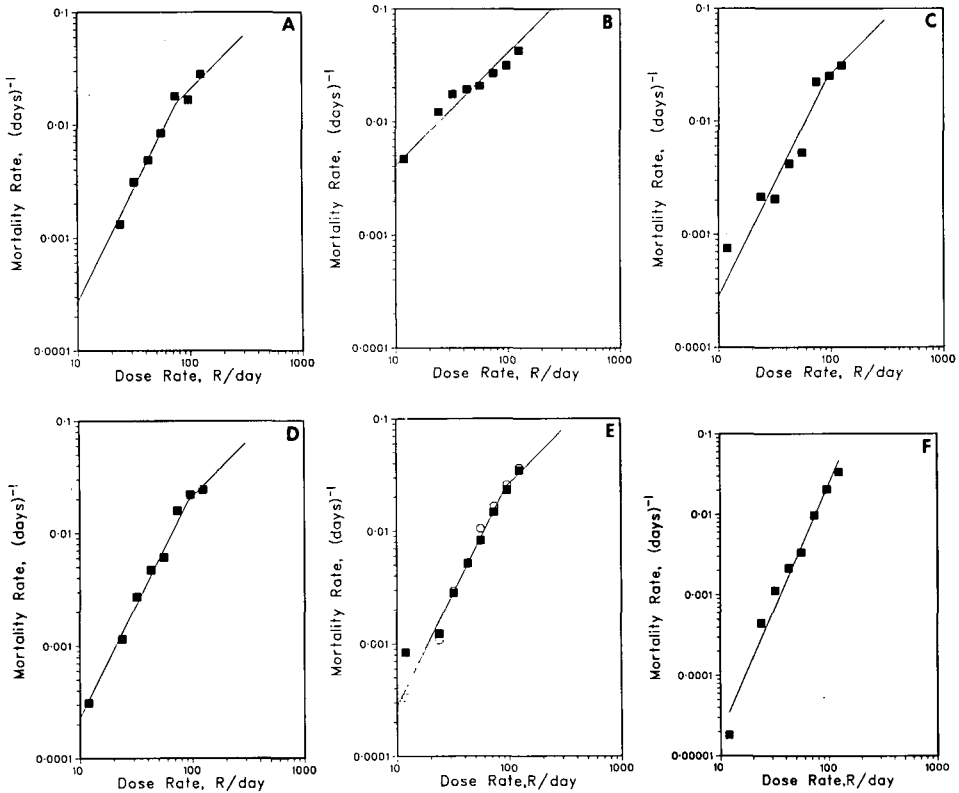


FIG.2. Death rate-dose rate relations for six rodent species. Two-branched lines fitted by least squares, except for black rat (2f), which has a least squares slope-intercept line. The taxonomic names are given in Table I. The species shown are: (A) chipmunk; (B) chinchilla (5 R/day point not included); (C) cotton rat; (D) house mouse; (E) multimammate mouse (two series); (F) black rat.

a_1 of Eq. 1' are those for which the total sum of squared deviations from each least square line is minimized, i.e. the estimates of a_2 and a_1 for which

$$S = \min \left\{ \sum_{k=1}^{N-1} [y_2(k) - Y_2(k)]^2 + \sum_{p=N-i}^N [y_i(p) - Y_1(p)]^2 \right\} \quad 2'$$

$$i = 1, 2, \dots, N-1$$

where $[y_j(\beta) - Y_j(\beta)]$ is the deviation from L_j , and N is the total number of points. Thus, the coordinates of the intersection of the selected set of lines characterize for each strain the transformed death rate-dose rate dependence. Their values are given (from Eq. 1') by

$$X' = a_1 - a_2; Y' = 2a_1 - a_2 \quad 3'$$

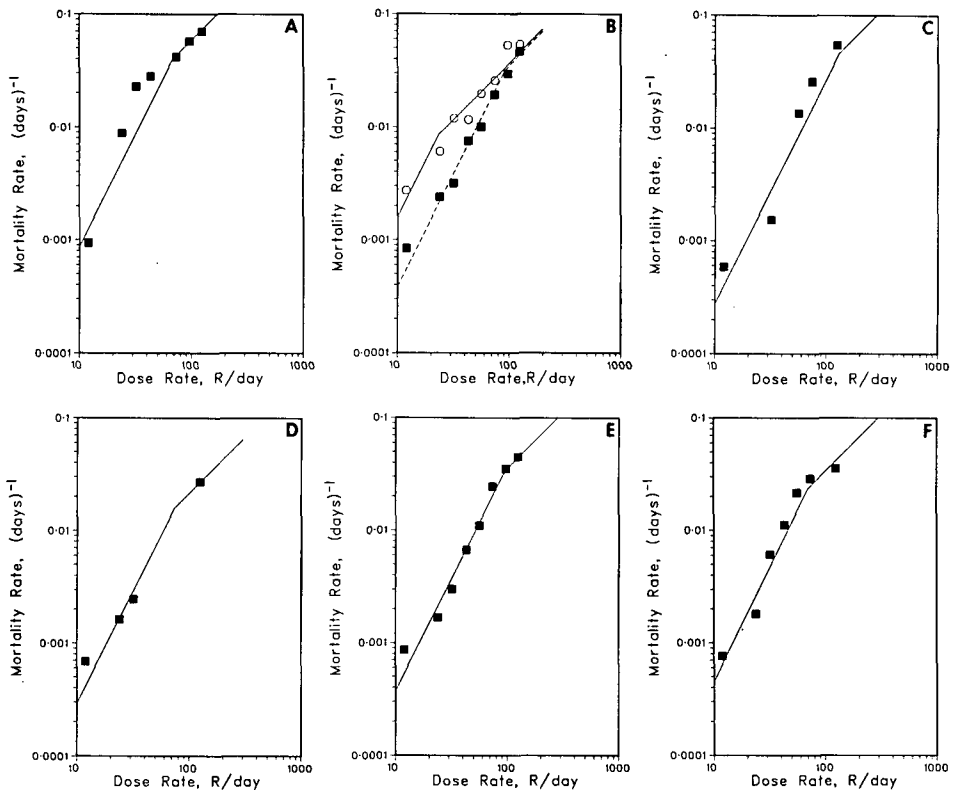


FIG. 3. Death rate-dose relations for six rodent species. Two-branched lines fitted by least squares. The taxonomic names are given in Table I. The species shown are: (A) rice rat; (B) California mouse, northern (○) and southern (■); (C) Florida mouse; (D) cotton mouse; (E) white-footed mouse; (F) harvest mouse.

The breakpoint coordinates for the laboratory mice are tabulated in Table II.

The wild rodents are a more heterogeneous group and also have the disadvantage that the highest daily dose given was only 125 R/day. In consequence, not all species show the complete two-branched response. Species with good slope-2, and good to weak slope-1 trends are: chipmunk, cotton mouse, white-footed mouse, harvest mouse, California mouse and multimammate mouse (Table II). The Florida mouse showed only the rate-squared trend, and the chinchilla showed a very extended slope-1 trend with no break to slope-2 even at 5 R/day.

The death rate-dose rate plots for dogs and guinea pigs were presented previously [10]. Both species agree well with the generic relationship, with well defined slope 2 and slope 1 branches. The goat also conforms to the two-branched function (Figure 4).

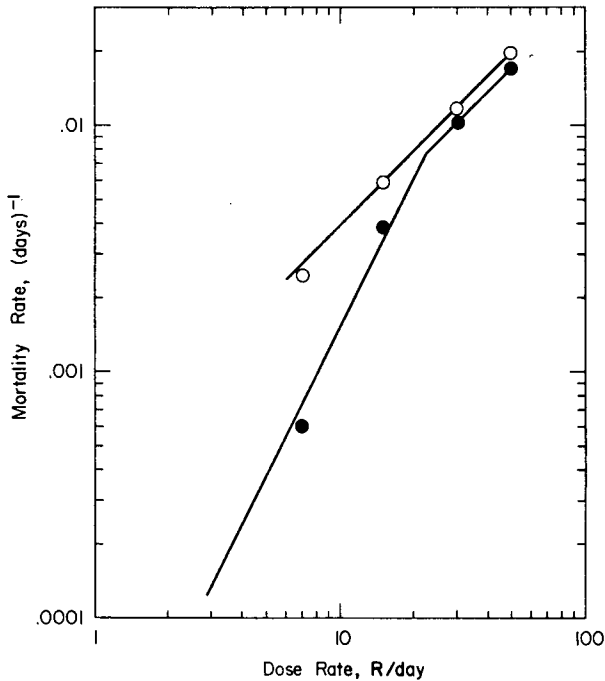


FIG.4. Death rate-dose rate relations for goats. Males, ○; females, ●. Data of Hupp et al. [13].

The data for the two subspecies of California mouse are graphed together in Figure 3b. They have the same slope-1 coefficient, but *P. c. insignis* breaks over to slope 2 at 91 R/day, like a typical cricetid rodent, while *P. c. parasiticus* remains on the slope-1 trend down to 24 R/day, so that it is much more radiosensitive than *P. c. insignis* on the slope-2 trend. A similar divergence between closely related populations is shown in the data for male and female goats [13] (Figure 4).

The two sets of symbols for the multimammate mouse, *Praomys natalensis*, (Figure 2e) are for a conventionally bred and maintained population (open hexagons) and a caesarian-derived, barrier-maintained population (solid squares). This difference in environments had no effect on the dose-rate dependence.

The black rat, *Rattus rattus*, Figure 2f, is the only case in which the mortality rates depart significantly from a slope-2 trend. The least-squares line fitted to the data has a slope of 3.08 ± 0.20 (Figure 2f). This is not a completely isolated instance, for laboratory inbred populations of the closely related Norway rat, *Rattus norvegicus*, given daily D.o.L. X-ray exposure [15] are comparable in radiosensitivity to mice or other rodents at high daily doses, but are considerably more radioresistant at low daily doses. This suggests that *R. norvegicus* may also have a slope greater than 2 at daily doses below the breakpoint.

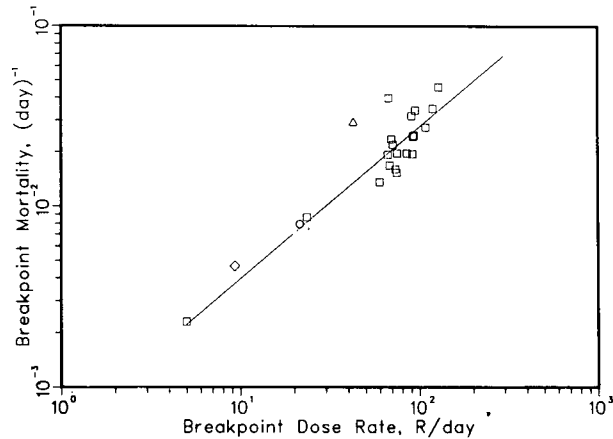


FIG.5. Coordinates of breakpoints for all species and genotypes in Table II except black rat. Data from Table II. Argonne wild rodents and laboratory mouse genotypes, \square ; dog, \triangle ; female goat, \circ ; guinea pig, \diamond .

The coordinates of the breakpoints given in Table II for the wild rodents, the laboratory mice, and the three additional species are plotted in Figure 5. The line through the data is a least-squares fit to the Argonne Laboratory wild and laboratory rodent data, with the coefficients

$$\log \rho = (0.840 \pm 0.083) \log r - 3.241 \pm 0.154 \quad (df=18) \quad 1$$

where ρ is mean radiation-specific death rate and r is daily dose in R/day. The breakpoints for guinea pigs, dogs and goats were not used to fit the line, but are in good agreement with the linear relation established by the Argonne rodent data.

A MATHEMATICAL THEORY FOR THE FORM OF THE DEATH RATE-DOSE RATE RELATION

The findings reported above are more definite and consistent than the earlier information about the dose-dependence for life-shortening in mammals [1, 2, 3, 4], and provide orientation for the development of a new mathematical theory of radiation mortality. Previous theories of radiation lethality were built on target theory considerations, but with no specific hypotheses about the nature of the target or the basis for its contribution to cell lethality. The consistent dependence of death rate on the square of the dose rate seen here is cogent evidence in support of a mathematical theory that was developed previously [16], based on the assumption that the killing of mammalian cells by ionizing radiations is due to cell injury and death brought about specifically by asymmetrical rejoinings of chromosome fragments arising from radiation-induced chromosome breaks.

ASSUMPTIONS AND DEFINITIONS

1. There are $C(t)$ cells in the population.
2. Each cell has m chromosomes, normally unbroken.
3. Radiation causes chromosome breaks. We assume that not more than one break occurs per chromosome because without this assumption the mathematical difficulties are enormous. With this assumption, only two kinds of fragments can arise:

proximal fragments (p -fragments), which contain the centromere;
 distal fragments (f -fragments), without the centromere.

Each p - and f -fragments has one 'hot' end, available for rejoining with another hot end.

4. The p - and f -fragments can rejoin in the forms pp , ff , and pf . We assume that asymmetrical pp and ff rearrangements are immediately lethal for the clone, whereas symmetrical pf rearrangements are fully viable.

5. The state, E_i , of a viable cell is determined by the (equal) number i of p - or f -fragments. All cells with unequal numbers of p - and f -fragments enter state E_0 , and are removed from the population.

6. Rejoinings occur at random, and all pairings of the fragments are equiprobable. The total number of ways in which one recombination may occur is $(2i \choose 2) = i(2i-1)$ (number of ways of forming groups of 2 from $2i$ elements).

Of these, i^2 are pf recombinations, since each of the i p -fragments may join with any of the i f -fragments. The number of pp or ff recombinations is $1+2+3+\dots+(i-1) = i(i-1)/2$. Thus

pp	pf	ff	Total
$\frac{i(i-1)}{2}$	i^2	$\frac{i(i-1)}{2}$	$2i^2 - i = i(2i-1)$

The proportion of viable rejoinings decreases with i as $i/(2i-1)$. For example, if $i=5$, 25 viable and 20 lethal pairings are possible for a total of 45.

TRANSITION PROBABILITIES DURING A SHORT INTERVAL

By assumption:

1. from state E_i to state E_{i+1} ($i=0, 1, 2, \dots, \mu-1$) the transition probability in a brief interval h is

$$\lambda Sh + \sigma(h);$$

here λ is a positive dose-effect constant with dimensions of reciprocal dose, S is the radiation dose rate, and $\sigma(h)$ is a residual that vanishes with h ;

2. from state E_i to state E_{i-1} ($i=1, 2, \dots, \mu$) the transition probability is

$$i^2 \gamma h + \sigma(h);$$

γ is a positive constant with dimensions of reciprocal time;

3. from state E_i ($i=2, 3, \dots, \mu$) to state E_0 the transition probability is

$$i(i-1)\gamma h + \sigma(h);$$

4. all other transitions have probability 0 or $\sigma(h)$.

Note that in this model no *direct* transitions can occur from E_0 or E_1 to E_δ (since no p - p or f - f recombinations are possible).

INITIAL CONDITIONS

Let $P_i(t)$ [or $P_\delta(t)$] be the conditional probability of finding the system in state E_i (or E_δ) at epoch t , given that it was in state E_0 at epoch 0. The initial conditions are

$$P_0(0) = 1$$

$$P_1(0) = P_2(0) = \dots = P_\mu(0) = P_\delta(0) = 0$$

THE DIFFERENTIAL EQUATIONS

The differential equations for the probabilities $P_i(t)$, $P_\delta(t)$, that a cell in the population is in state E_i or E_δ are:

$$\frac{dP_0(t)}{dt} = -\lambda SP_0(t) + \gamma P_1(t) \quad 2$$

$$\frac{dP_i(t)}{dt} = -[\lambda S + i(2i-1)\gamma]P_i(t) + \lambda SP_{i-1}(t) + (i+1)^2 \gamma P_{i+1}(t) \quad 3$$

$i = 1, 2, 3, \dots, (\mu-1)$

$$\frac{dP_\delta(t)}{dt} = \sum_{j=2}^{\mu} j(j-1)\gamma P_j(t) \quad 4$$

$$\frac{dP_\mu(t)}{dt} = \lambda SP_{\mu-1}(t) - \mu(2\mu-1)P_\mu(t)\gamma \quad 5$$

It should be emphasized that the constant μ ($\mu < m$) is *not* a parameter of the system, but rather an upper limit to the number of breaks per cell that can occur if the error arising from the assumption of not more than one break per chromosome is to be kept within an acceptable limit. To limit this error, $P_\mu(t)$ must be less than an assigned value ϵ for all values of t .

The set of equations, Eq. 2-5, was integrated numerically for a series of dose rates, S , with two values of the dose-effect constant λ and three values of the rate constant γ . Figure 6 shows the stationary death rate attained in populations of cells given constant exposure at rate r . The death rate, ρ_∞ , computed from the relation

$$\rho_\infty = \frac{1}{P_\delta} \frac{dP_\delta}{dt} \Big|_{t=\infty} \quad 6$$

reaches an asymptotic value for time sufficiently long so that $\gamma t \gg 1$.

Figure 6 gives the relation of ρ_∞ to r for four combinations of λ and γ . When λ is held constant and γ varies, we get a family of curves that approach a common asymptotic line of unit slope. At low dose rates each curve becomes asymptotic to a straight line of slope 2. The point of intersection of the asymptotic branches of slope 1 and slope 2 for a specific curve has the coordinates γ on the y (death rate) axis and γ/λ on the x (dose rate) axis.

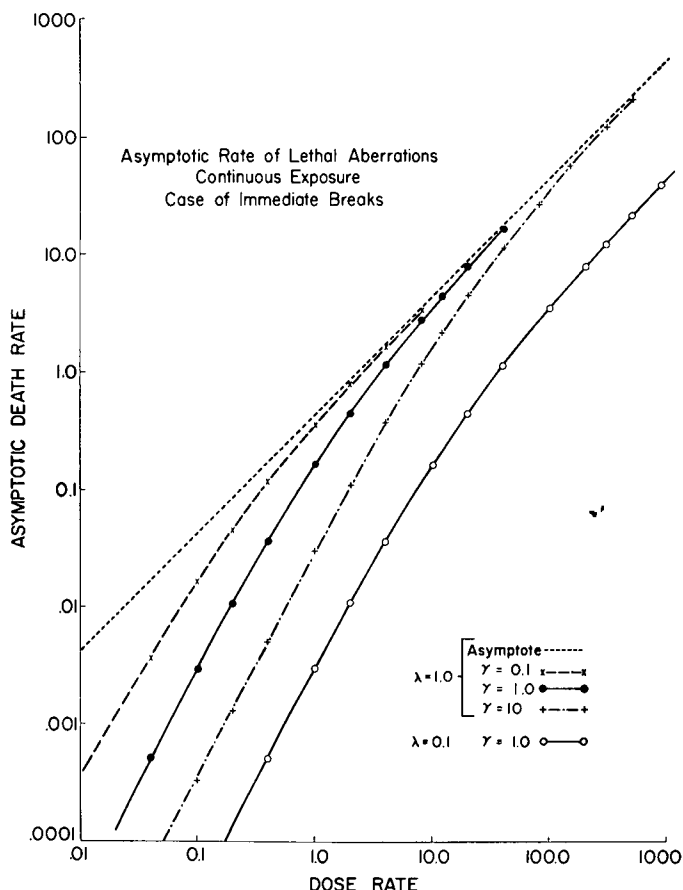


FIG. 6. Relation of death rate to dose rate for a population of irradiated cells computed from the mathematical model given in the text. Computed for four sets of parameters, as specified in the key. The dashed line of unit slope is the common asymptote for the three curves with $\lambda = 1$.

The locus of intersections for all curves with constant λ and varying γ is the common upper asymptotic line of unit slope. If γ is held constant and λ varies, the locus of intersections is a line of zero slope, i.e. the curves are shifted laterally.

This mathematical model predicts the hyperbolic form of the death rate-dose rate function, and the asymptotic slopes. The model does bend over from slope 2 to slope 1 more gradually than do the experimental data. This is due in part to the existence of a latent time to death at high doses or dose rates, arising from the finite survival time of postmitotic differentiated cells [15, 9]. The gradual transition from slope 2 to slope 1 in the model is also due to the assumption of no more than one break per chromosome. If multiple breaks are allowed, damage accumulates more rapidly with dose rate above the intersection point, and the transition to slope 1 is more abrupt.

The experimental breakpoints are equivalent to the intersections of the asymptotes in the model. It was shown above that the breakpoints for the Argonne rodent series lie on a straight line trend with a slope 0.84, not significantly lower than the line of unit slope that is predicted by the model if all species have the same dose sensitivity coefficient, λ . The three additional species also conform to the fitted line. The small departure from unit slope could be due to a correlation between λ and γ across species, but we consider it premature to draw any such conclusion at this time, because the theory is still in an early stage of development, and important factors have not yet been taken into account.

The ratio of the x and y coordinates of a point on the slope-1 branch is an estimate of the dose-effect constant, λ . Table II lists the λ values so estimated for the eight laboratory mouse genotypes and for the 12 wild rodent species. The overall mean is $(280 \pm 17 \times 10^{-6} \text{R}^{-1})$, with a coefficient of variation (CV) of 30% ($df = 17$). The laboratory mice, with average λ of $260 \times 10^{-6} \text{R}^{-1}$, do not differ significantly from the wild rodents.

The time constants, γ^{-1} , for the experimental populations are given in Table II, estimated as the reciprocal of the y -coefficient of the breakpoint, which is equivalent to the y -coefficient of the intersection of the asymptotes in the model. The γ^{-1} values range from 22 days for the Florida mouse to 115 days for the northern California mouse. No breakpoint was observed for the chinchilla, but the smallest value of γ^{-1} it can yield is 435 days. The time constants for guinea pigs and goats are also well above 100 days. There is, then, more than a tenfold range of γ among mammalian species. This implies a variation between species by a factor of 10 or more in the dose rate needed to attain a given degree of life shortening at low dose rates, or a variation by a factor of 100 or more in the amounts of life shortening produced by a given dose rate, if there is no species variation in the dose-effect coefficient.

The time constant γ^{-1} is an order of magnitude greater than any time constant encountered heretofore in radiation biology. The time constant for repair of damage to cultured mammalian cell populations given split doses, which is related to repair events that occur within a single cell cycle, is on the order of a few hours at most [17]. The time constant, θ , for the sparing effect of fractionated exposure in producing lifespan shortening, which may be related to the kinetics of growth in tissue cell populations [18], is about 5 days for mice [1]. As yet there is no clue to the biological nature of the gamma term. We suggest that it is not a repair time, but rather an *accumulation time*, i.e. the time interval over which damage events in DNA leading to chromosome breaks can accumulate in a cell population and be available for interaction with other damage events. The long times involved--22 days to more than 500 days--suggest that this is the cell population in the hematopoietic tissues that turns over most slowly, and the stem cell population immediately comes to mind. This hypothesis is accessible to test by means of procedures that alter the turnover of cells in the stem cell compartment.

IMPLICATIONS FOR THE ESTIMATION OF THE HUMAN HAZARD

There is no significant direct contribution of the rate-squared term to the human hazard at near-background exposure levels. If, for example, γ^{-1} for man is taken to be 3650 days (on the assumption that γ^{-1} increases with lifespan), and λ to be $600 \times 10^{-6} \text{R}^{-1}$, the excess mortality for an

incremental dose commitment of 35 mR/yr (about 50% increase over natural background) is about 10^{-11} (days) $^{-1}$. The mean death rate from natural causes for the human population is 4×10^{-6} (days) $^{-1}$, so the expected fractional lifespan shortening would be 0.00025%, or less than a tenth of a day in 70 years. Change of either λ or γ^{-1} by a factor f would alter this estimate of excess mortality by a factor of f^2 .

The problems of environmental radiation hazard for man is, therefore, governed by the magnitude of the term for damage that accumulates as the first-power of the dose. This term becomes dominant at very low dose rates.

In earlier papers [19, 20] it was shown that when the B6CF₁ hybrid mouse was given daily exposure down to 0.3 R/day the mortality rate slope changed to first power at the lowest daily doses, with a crossover point (breakpoint) at about 18 R/day. The dose-effect constant, denoted α (alpha), for the B6CF₁ mouse was $65 \times 10^{-6} \text{R}^{-1}$. This is one-fourth as large as the mean λ value of $260 \times 10^{-6} \text{R}^{-1}$ for laboratory mice (Table II).

Previous studies by Lorenz et al. [11] on mice given radium gamma rays at daily doses up to 8.8 R/day, and by Boche [14] on rats given X-rays at daily doses up to 10 R/day had established the existence of the alpha term for these two closely related species. Up to this time, an alpha term has not been identified for any other species, but it is prudent to postulate that every species has an alpha term. Work is now proceeding at Argonne National Laboratory in an effort to estimate the alpha term for beagle dogs given D.o.L. exposures at 0.4 to 5 rad/day [10], and white-footed mice exposed at 3.6 to 16.7 rad/day 5 days per week [J. F. Thomson, personal communication].

It was shown previously [10] that the rate-squared trends for both guinea pig and dog intersect the alpha branch for the mouse without showing any indication of breaking over to the alpha trend. Therefore, these two species, which have high acute radiosensitivity, nevertheless have alpha terms equal to, or perhaps less than, that for the radioresistant mouse. This suggests the alternative hypotheses that mammals in general have alpha terms (a) equal to or (b) less than or equal to the mouse value. We are unable as yet to decide which of these alternatives holds, but the data and theory presented above should help to decide the issue eventually.

Mechanistically, the alpha term can be a function either of (1) radiation-induced damage entirely, or (2) radiation-induced damage interacting with 'spontaneous' damage, i.e. chromosome breaks that occur independent of radiation exposure. In the former case, the single-hit events contributing to the alpha term should be, at least in part, of the same physical nature as the individual damage events leading to chromosome breaks and rearrangements. Furthermore, whatever DNA repair capacity is operative in a given species, repair should be equally efficacious regardless of whether one damage event is produced in a cell (alpha damage) or two or more events per cell (lambda damage). If this is so, then the α coefficients would be expected to be roughly proportional to the λ terms across species, and to have about the same CV. According to this, most mammalian species would have α terms lying within a twofold range of variation, in an interval containing the value of $65 \times 10^{-6} \text{R}^{-1}$ that was found for the B6CF₁ mouse.

The small interspecies variance of λ suggests that there is comparatively little interspecies variance in the efficacy of repair of DNA damage produced by ionizing radiations. This is consistent with the finding of Hart and Sacher [21, 22] that *Mus musculus* and *Peromyscus leucopus*, which differ in

lifespan by a factor of 2 (Table I) and have a 2.5-fold difference in repair of UV lesions in DNA, do not differ in the rate or amount of repair of DNA produced by ionizing radiation.

According to the second hypothesis, the alpha term is due to two-break events in which one radiation-induced break interacts with a 'spontaneous' break. The governing factor is then the average frequency of spontaneous breaks in the cells of each species. We do not at present have quantitative estimates of that frequency for our experimental species and man, beyond the plausible hypothesis that the prevalence of spontaneous breaks is negatively correlated with species lifespan and capacity for repair of DNA damage leading to spontaneous breaks. On that basis, long-lived animals might be expected to have lower alpha values than those measured for rats and mice. The Argonne experiments with beagle dogs and white-footed mice are critical for a decision about this hypothesis.

The value of γ for the human species must be considered to have a factor of 10 uncertainty. Table II shows that the large and long-lived species tend to have larger γ^{-1} values than the small and short-lived. It is possible, therefore, that *Homo sapiens* has γ^{-1} of hundreds, or even thousands, of days. This is a matter of great concern, for it raises the possibility that radiation damage accumulated in human chromosomes over this long span of time can interact to produce lethal rearrangements.

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DISCUSSION

Y. NISHIWAKI: In some of your graphs of mortality rate as a function of dose rate, the line appears to have been drawn quite arbitrarily with only a few experimental points. In view of the possible range of statistical variation of the data, I should have thought it would have been rather difficult to obtain consistent values for the breakpoint mortality or breakpoint dose rate. This is a very interesting analysis, but how could we estimate the breakpoint mortality or breakpoint dose rate more objectively? Even if the least-squares method is used, I wonder whether a slight change in the angle of the line might not cause some difference in the estimation of the breakpoint mortality or breakpoint dose rate.

G.A. SACHER: Our death rate versus daily dose representation was developed after the data collection was completed, and it was not possible to extend the dose range in the several instances where the upper or lower branches were established only weakly or not at all. All the breakpoint values reported in the tables as definite values, rather than inequalities, were estimated by the least-squares procedure described in the text; this procedure is objective and was in fact executed by a computer program. In those instances where one branch is determined by a very few points the sampling error is large, but this can be avoided in future studies by a judicious choice of treatment levels. There is no problem arising from a slight change of angle because the fitting procedure is based on the assumption that the two branches have slopes of 1 and 2 exactly. In only one case, the black rat, was this assumption rejected at a high level of significance. It should be noted that in the other cases where there was an apparent poor fit the data were based on very small numbers of animals.

Y. NISHIWAKI: In your analysis, the dose per day is used as a measure of dose rate. If a pulsed beam is used for irradiation, the dose rate of this beam would be very high, even if the average dose per day were low. In this case, would the high dose rate of the pulsed beam affect the result?

G.A. SACHER: The 'dose rates' referred to in the text are in fact daily dosages based on exposures ranging from 22 hours per day down to a few minutes per day. Since the effectiveness of low-LET radiation depends strongly on the exposure time, corrections should in principle be applied to the daily dose, to allow for such differences. However, such changes in ultrafractionation or protraction time should not alter the basic quadratic relationship.

Y. NISHIWAKI: What would be the effect of a change in LET on the breakpoint dose rate? You state the radiation to be low-LET; I should like to know up to what value of LET the radiation is considered to be low-LET for the purposes of this analysis.

G.A. SACHER: I am aware that variations of LET in the range from 200 kV X-rays to ^{60}Co gamma rays do not alter the two-branched relationship. On the other hand, fast neutrons yield a death rate versus daily dose plot that has slope unity over a range of mean mortality rates for which the same mouse strains would be almost entirely in their slope-2 range. Since this was work done by Evans and Henshaw in the 1940s, before absolute neutron doses could be measured, I have no certain knowledge about the rad or kerma doses involved. However, I believe that we can infer that high-LET radiations either abolish the slope-2 branch or displace it to the left by an unknown amount (see Ref. [20]).

H. WIJCKER: Was the breakpoint concept the starting point which was confirmed by analysis with linear and quadratic curves or was it the other way round, i.e. with the breakpoint concept constructed on the basis of the linear and quadratic parts observed in the curves? If the latter, I wonder whether the set of points could not be the expression of a sigmoidal curve which might be approximated by a quadratic function in the curved part bending up and by a linear part around the inflection point. Have you looked into this possibility?

G.A. SACHER: The death rate versus dose rate representation for experimental data was developed first, and the mathematical theory was evolved to explain the two-branch relation in which the lower branch was seen to be almost exactly slope 2, and the upper branch approximately slope 1. The model was developed around the assumption that chromosome rearrangements are the lethal event, because that model yields both the slope-2 trend at low dose rates and a bending over to a saturation, slope-1 branch at higher rates.

A sigmoid curve, such as any of several growth curves, could undoubtedly be fitted to the data, but it must be pointed out that the specifically slope-2, slope-1 property would not be necessary features of these models, and would have to be introduced by further specific assumptions. I do not know what the nature of these assumptions might be, or how they could be related to observable biological entities and processes. We know that radiations produce chromosome breaks, that a variety of chromosome rearrangements are injurious to the mitotic process, and that these rearrangements require two breaks. These were for me sufficient reasons to examine a falsifiable hypothesis about the real process of chromosome aberrations, rather than an abstract 'target' model.

H.H. VOGEL: In your excellent analytical paper you have summarized data from gamma irradiation of more than 20 mammals with a nice theoretical interpretation. Does your hypothesis give a rational (and testable) theory to explain the major differences in the response of male and female goats to gamma radiation? I should think you might find a difference in the marrow stem cell between the sexes of this species. Or could the difference be endocrine?

I should like to call your attention to the experiments on gamma irradiation of large mammals carried out over the years at the Comparative Animal

Research Laboratory (CARL) at Oak Ridge, Tennessee. Some of these have involved fractionated and protracted exposure of pigs, sheep, cows, goats, horses, etc. I should think that these data, if available, would be a useful supplement to your own data from large mice. Also you could probably obtain some primate data from the Armed Forces Radiation Research Institute at Bethesda, Maryland.

G.A. SACHER: The biological nature of the accumulation time, γ^{-1} , is not deducible from any information known to me. Because of its magnitude, I put forth the conjecture that it is related to the turnover time, or perhaps time out of cycle, for the haematopoietic stem cell population. The conjecture will stand or fall on the outcome of measurements of stem cell parameters and survival parameters in critical situations such as Hupp's male and female goats, or my two subspecies of *Peromyscus californicus*.

COMBINED EFFECTS

Session 9, Part 1

IN VITRO CULTURE OF PRE-IMPLANTED MOUSE EMBRYOS

A model system for studying combined effects

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Abstract

IN VITRO CULTURE OF PRE-IMPLANTED MOUSE EMBRYOS – A MODEL SYSTEM FOR STUDYING COMBINED EFFECTS.

Studies on combined effects, e.g. interaction between chemical toxicants and ionizing radiation, are difficult to perform, as they are dependent on many factors (substance concentration, radiation dose, sequence of treatments, etc.). In order to obtain data from such studies it is necessary to establish a comparatively simple experimental model system. An in vitro system should be preferred on the one hand, as an exact determination of the substance concentration and the timing of the incubation period are possible. On the other hand the system should be a mammalian system very similar to in vivo conditions. We have established such a model system by studying combined effects on pre-implanted mouse embryos cultured in vitro. This system has the following advantages: (1) The embryos can be cultivated for several days in vitro; (2) Their physiological intactness can be tested; and (3) Cell proliferation, cell killing and chromosomal damage can be investigated comparatively easily. The embryos are isolated at the 2-cell stage and incubated in a culture medium in vitro. The development of the embryos is followed under the microscope until the development of blastocysts or the hatching of blastocysts is observed. These blastocysts can be transplanted to fostered mice and the development of normal animals determined. The proliferation kinetics can be studied easily, and the methods are described. A method has also been developed to measure the DNA content of individual cells by microscope fluorometry. After treatment of the embryos with ionizing radiation or drugs the release of micronuclei has been observed from the cell nuclei, which is an expression for chromosomal damage. Thus cytogenetic effects can be evaluated. Substances or radionuclides can be added to the culture medium or external irradiation can be performed during the culture period. Also the combined effects of radiation and heating can be studied. The effects of X-rays and tritiated compounds have also been investigated. The combined effects of radiation with antibiotics such as actinomycin D, and environmental toxicants such as lead, have been determined. The system described has been useful to evaluate cytological, teratogenic and cytogenetic effects. Toxicological effects can be demonstrated. The model system is recommended for screening combined effects and investigating interaction mechanisms.

INTRODUCTION

When a cytotoxic substance (a concentration with the effect S) is combined with ionizing radiation (a dose with the effect R) various types of interaction effects (JE) can result. The combined effect can be higher than the addition of both single effects ($JE = S + R$), and this situation is also called synergistic, potentiating or multiplicative. The effect can be additive ($JE = S + R$), it can be less than additive ($R - JE - A + B$), or even an antagonistic effect can result ($JE - R$). Furthermore, substances which have no cytotoxic effect can enhance the effect of ionizing radiation. For such a mechanism oxygen is a well-known example.

Combined effects are influenced by multiple factors. The interaction between external radiation and a chemical substance is dependent on the time sequence of treatment as well as the interval between the administration of both agents; it is dependent on the dose of the substance as well as the radiation; it is dependent on the distribution and the metabolism of the substance, and so forth. The situation becomes even more complicated when combined effects between incorporated radionuclides and substances are studied.

In order to investigate some of the factors mentioned it appears of advantage to use a comparatively simple experimental model system. On one hand it is preferable to work with an *in vitro* system, as an exact determination of the substance concentration and of the timing is possible. On the other hand one should work with a mammalian system which has features very near to *in vivo* conditions.

We have established such a model system by studying combined effects on pre-implanted mouse embryos cultured under *in vitro* conditions, and observing the development of the embryos as well as other cellular processes. This system has apparently the following advantages:

- (1) The embryos can be cultivated for several days *in vitro* with the advantages mentioned.
- (2) The physiological intactness of the developed embryos can be tested *in vivo*.
- (3) Effects on cell proliferation with its various parameters, on cell killing and on chromosomal damage can be studied.

A disadvantage of such a model system is that effects can only be measured when they occur within some days after irradiation or other treatment. Furthermore, metabolic processes are included to a limited degree only, although the substance concentrations are better defined than with *in vivo* conditions. About six generation cycles and the proliferation with DNA-replication can be followed. Also damage to the genetic material can be measured. The findings should therefore have some meaning for late effects also, and can be used as a screening system.

CULTURE OF PRE-IMPLANTED MOUSE EMBRYOS

One male and two female sexually mature mice were mated for three hours. Females with vaginal plugs were segregated and the embryos were obtained from these animals about 30 hours later by flushing culture medium BMOC-2 [1] through the oviduct. At this time the ova are in the 2-cell stage surrounded by the zona pellucida (Fig. 1). These embryos are cultivated by the method of Brinster [1] with some minor modifications as described previously [2].

The development of the embryos in the culture is followed under the microscope. In the untreated cultures 48 h after conception (p.c.) the embryos are in the 4-cell to 8-cell stage with an average cell number of 6.0 cells per embryo; 72 h p.c. they are in the stage of morulae (about 21 cells per embryo); 96 h p.c. some embryos have developed to blastocysts (about 64 cells per embryo); 120 h p.c. 92% of the embryos have developed to blastocysts or are already hatching from the zona pellucida (Fig. 2); 144 h p.c. 86% of the embryos have hatched after having developed to blastocysts. The average cell number is 96 per embryo at this stage.

When the unhatched blastocysts are transplanted into fostered female mice, they hatch in the uterus, implant and develop to normal animals to an extent of 29% of the blastocysts which have grown in untreated cultures. The in vitro grown blastocysts are apparently physiologically intact and this can be tested by the in vivo development.

During the in vitro culture the mouse embryos can be irradiated by X-rays or other radiation sources. Chemical substances as well as radionuclides can be inserted into the culture medium and can be removed again by changing the medium. All these manipulations can be performed at the various defined developmental stages and partly also at defined phases of the generation cycle.

DETERMINATION OF PARAMETERS OF CELL PROLIFERATION

Up to a cell number of 8 per embryo the cell boundaries can be observed under the microscope. In the later developmental stages the embryos are treated on a slide with a 1.0% Na-citrate solution [3].

By the hypotonic treatment the embryos and the cell membranes are destroyed, the isolated cell nuclei are fixed on the slide and their number can be counted after staining according to Pappenheim (Fig. 3). Also the mitotic index can be determined under these conditions.

When ^3H -thymidine (2 $\mu\text{Ci/ml}$; sp. act. 48 Ci/mmol) is added directly before the hypotonic treatment for 15 minutes to the culture medium, the cell nuclei, in which DNA synthesis was performed during this period, appear labelled after autoradiography (dipping in gel emulsion, Ilford K2; exposure for 3 weeks) (Fig. 3). Thus the labelling index can be determined.

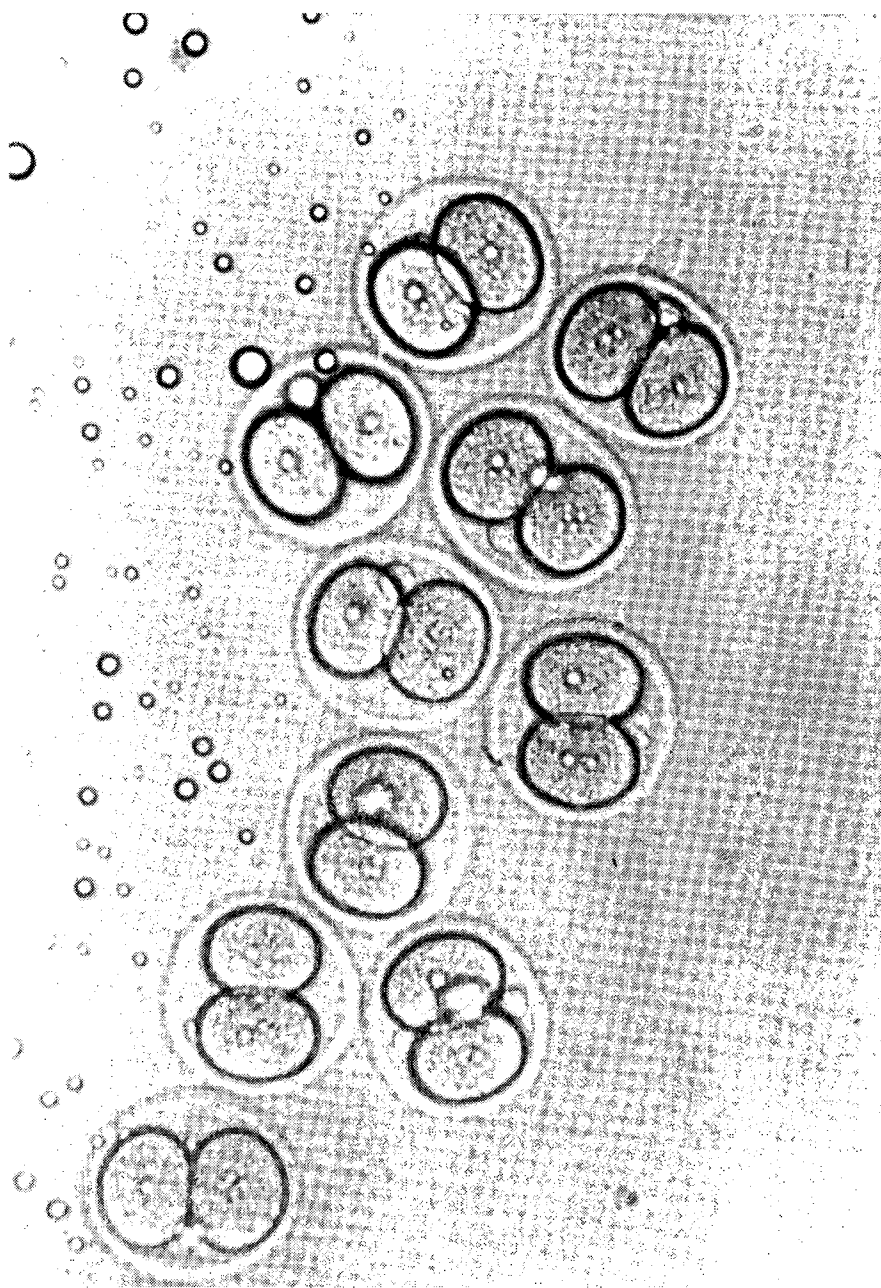


FIG.1. Pre-implanted mouse embryos in the 2-cell stage (two cells per embryo are surrounded by the zona pellucida).

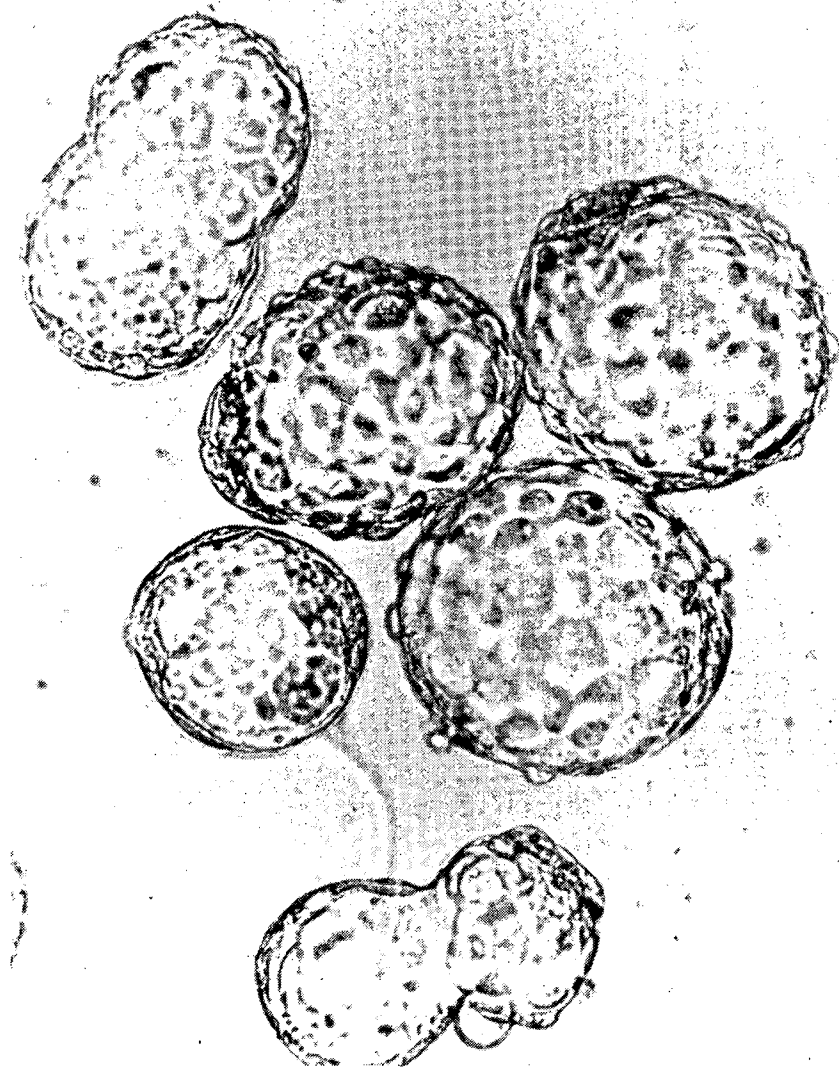


FIG.2. Pre-implanted mouse embryos after in vitro culture to blastocysts. Some of the blastocysts are hatching from the zona pellucida, some have already hatched completely. One 'empty' zona pellucida is seen also.

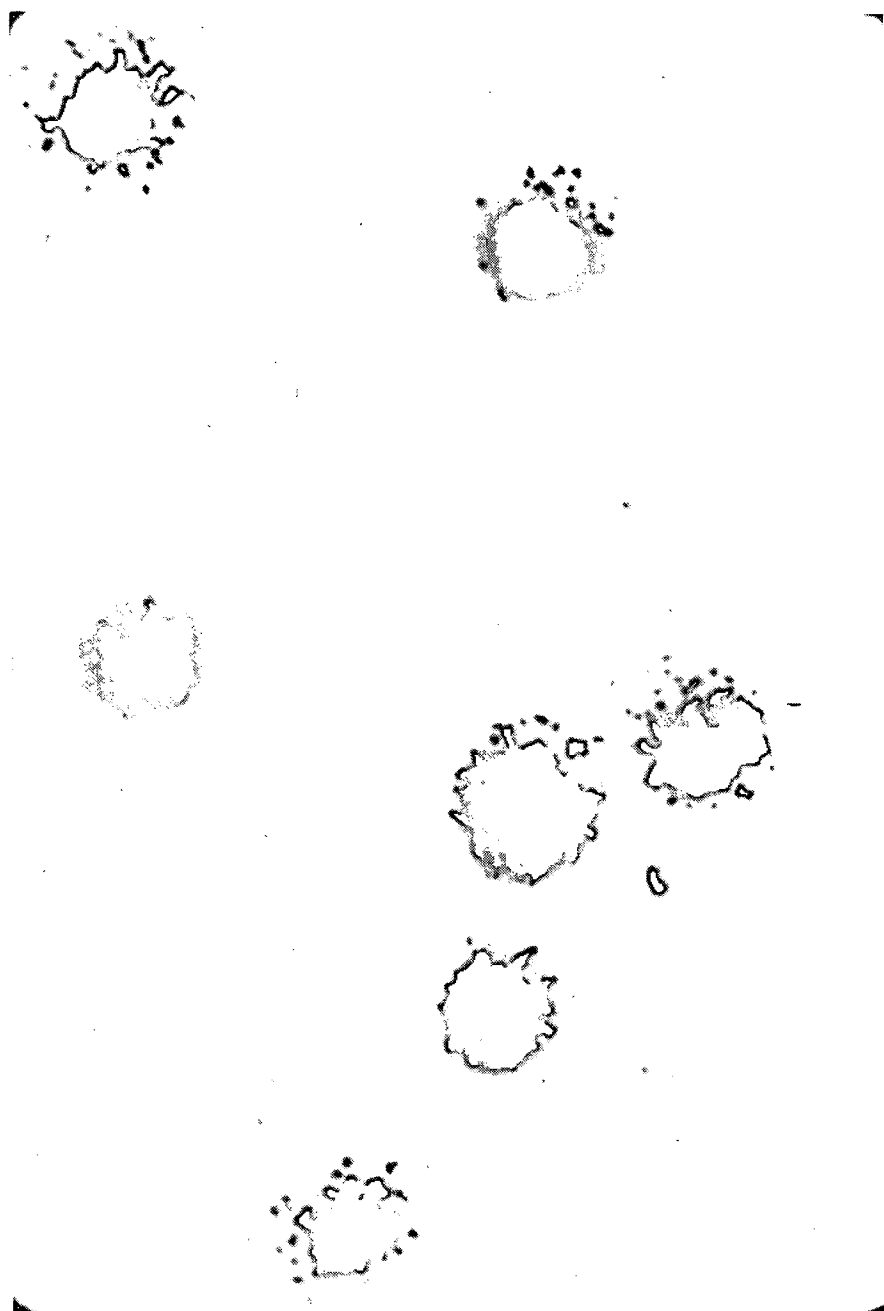


FIG.3. Isolated cell nuclei of pre-implanted mouse embryos after incubation of the intact embryo with ^3H -thymidine and autoradiography. Six nuclei are labelled, one is unlabelled (labelling index is 86%).

In the same nuclei the individual DNA content can be measured with a microscope photometer (MPV 1, Leitz, Wetzlar, Fed. Rep. Germany). The nuclei are stained with ethidium bromide [4] and the fluorescence is measured at 530 nm. By the combination of these methods and of the data obtained, the various parameters of proliferation kinetics such as cell cycle times and duration of the various phases can be determined.

Combined effects between ionizing radiation and chemical substances are interesting from two aspects. On one hand, in cancer therapy it is desirable to sensitize tumour cells in such a way that the relative radiation damage is larger in the tumour than in normal tissues. On the other hand, in estimating radiation risks living organisms including man are very frequently exposed not only to radiation alone but simultaneously also to toxic substances such as environmental pollutants. The resulting effects can be less than additive, additive or even more than additive (synergistic) in comparison with single exposures.

The experimental experience is much larger for combined effects with chemotherapeutic drugs in tumour therapy, and much can be learned from these data for the interaction of radiation with pollutants and other toxic substances with respect to risk estimates.

COMBINED EFFECTS OF ACTINOMYCIN D AND RADIATION

The radiosensitizing effect of actinomycin D has been studied frequently [5–7]. Elkind and co-workers [8] have observed that intracellular recovery from sublethal radiation damage is impaired when actinomycin D is added to mammalian cell cultures during or shortly after irradiation. These findings are important for combined treatments in radiotherapy [9], where the combination of actinomycin D and radiation has been studied for treatment of cancers [10].

We have investigated this combined effect on the development of the pre-implanted mouse embryo. The radiation source was tritium in the form of tritiated water. Actinomycin D as an inhibitor of RNA synthesis blocks per se this development without irradiation [11]. When the culture medium contained 5×10^{-2} $\mu\text{g/ml}$ actinomycin D, 16% of the embryos developed to blastocysts (96% in the controls). However, when the concentration was only 10^{-4} $\mu\text{g/ml}$ actinomycin D the development of the embryos was not different from the untreated controls. This concentration of actinomycin D was used for the further experiments with tritiated water.

After incubation of the embryos with tritiated water the development to blastocysts decreased with increasing tritium concentrations [12]. No blastocysts were observed at a concentration of 200 $\mu\text{Ci } ^3\text{H-H}_2\text{O}$ per ml medium. The same effect was already found with 100 $\mu\text{Ci/ml}$ tritium, when the culture medium contained 10^{-4} $\mu\text{g/ml}$ actinomycin D. Actinomycin D, when given in such a

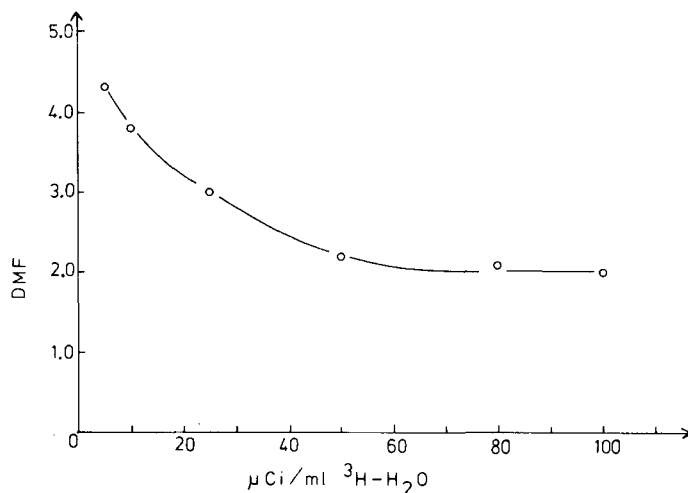


FIG.4. Dose modifying factor (DMF) of 10^{-4} μg actinomycin D per ml culture medium. The effect of tritiated water on the development of pre-implanted mouse embryos was compared after incubation with and without actinomycin D.

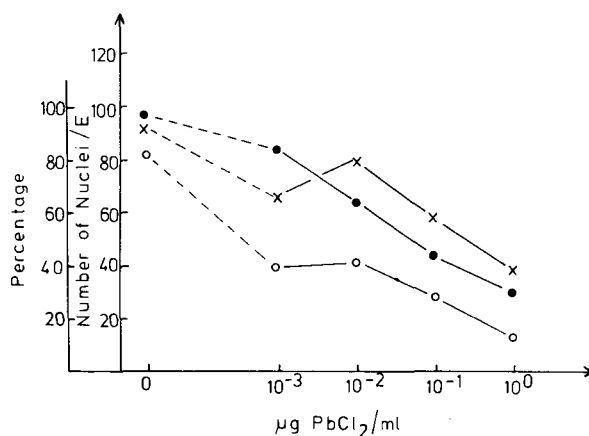


FIG.5. The development of pre-implanted mouse embryos to blastocysts 96 hours p.c. (X, percentage); hatching of blastocysts 144 hours p.c. (O, percentage) and number of cell nuclei per embryo after in vitro culture with various concentrations of PbCl_2 .

concentration alone, had no effect at all on the embryo development, as mentioned above. Thus the combined effect of the antibiotic and radiation is higher than additive under these conditions. The interaction is dependent on the dose of the substance as well as of the radiation.

The dose modifying factor (DMF) (in analogy to RBE the dose ratio at equal biological effects) for the above-mentioned conditions is 2.0. When the radiosensitizing effect of actinomycin D was measured at lower radiation doses (smaller tritium concentrations), the dose modifying factors calculated for equal biological effects increase (Fig. 4).

This is apparently a characteristic feature observed for radiosensitizing substances which influence repair and recovery processes [6, 7]. As intracellular recovery is small after exposure with high LET radiation, the dose dependence of DMF is therefore mainly found with low LET radiation. As the recovery is more significant at low radiation doses or after irradiation with low dose rate, a modification of such processes plays a greater role under these conditions. An analogous dependence with a similar mechanism is found for the RBE of high LET radiation [13]. This may have some practical influence on risk estimations. However, one has to keep in mind that an increase in the DMF appears, as the reference effect (radiation alone) does not show a 'linear' dose effect relation.

COMBINED EFFECTS OF LEAD AND RADIATION

As an example of an environmental pollutant, lead and its effect on pre-implanted mouse embryos were studied in combination with X-rays. A number of substances which react with sulphydryls and other nucleophilic groups such as N-ethylmaleimide [14], iodoacetamide [15] and mercury compounds [16] are known to act as radiosensitizers [6, 7]. Lead ions form complexes with nucleophilic groups of proteins and nucleic acids in the same way as mercury ions. Therefore one can expect that lead ions are also radiosensitizing.

Figure 5 represents data for the embryo development to blastocysts, for the hatching of blastocysts and the number of nuclei per embryo in dependence on the PbCl_2 concentration in the culture medium. Very small concentrations (10^{-3} $\mu\text{g/ml}$) show already an effect on all three parameters. The best dose correlation is observed for the number of nuclei per embryo. However, measurements of Pb^{2+} concentration in the medium after about 110 hours incubation (144 hours p.c.) demonstrated that the lead content changed in the medium probably due to adsorption to the material of the petri dishes, for instance, when the smaller PbCl_2 concentrations were used. In order to obtain better reproducible results only PbCl_2 concentrations of 0.1 μg and 1.0 μg PbCl_2 per ml culture-medium were used in the following experiments.

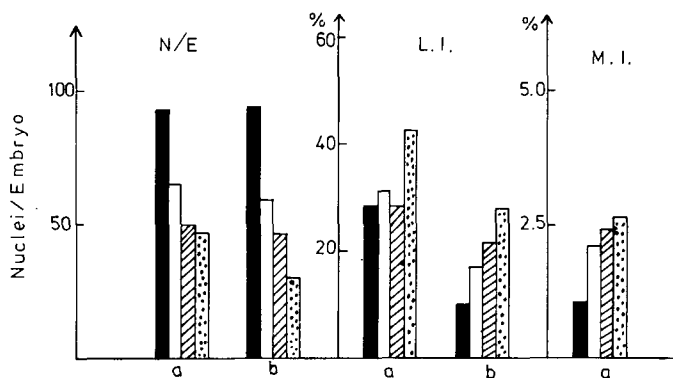


FIG. 6. Number of cell nuclei per embryo (N/E), labelling index (L.I., percentage) and mitotic index (M.I., percentage) in pre-implanted mouse embryos after *in vitro* culture (a) 120 hours p.c., (b) 144 hours p.c.; ■ untreated controls; □ X-irradiation with 100 R; ▨ incubation with 0.1 μg PbCl_2 per ml culture medium; ▩ combined treatment.

As described above, in untreated control cultures 86% of the embryos developed to blastocysts and hatched. This value decreased to 66% when the embryos were irradiated with 100 R 240 kV X-rays at the 2-cell stage, and to 28% when the embryos were incubated with 0.1 μg per ml PbCl_2 . After combining X-irradiation and incubation with lead, only 13% of the embryos developed to blastocysts and hatched.

Similar data were obtained by determining the number of cell nuclei per embryo (Fig. 6). Especially 144 h p.c. the effect of the combined treatment was larger than that of the single treatments. A considerable cell loss apparently occurred under these conditions from 120 h to 144 h p.c. At the same time DNA synthesis expressed by the labelling index after incubation with ^3H -thymidine increased significantly for the combined treatment. Also the mitotic index was enhanced (Fig. 6). Thus the biological system apparently tries to compensate for the damage, however without success.

In further experiments the DNA content of each individual cell nucleus was measured by microscope fluorometry 72 h p.c. (about 40 h after starting the incubation). In the untreated control cultures the embryos have developed at this time to morulae with about 21 cells per embryo. About 66% of the cell nuclei were labelled by ^3H -thymidine, and showed a DNA content which was expected for S-phase cells. Thus good agreement was found between both methods which was also checked for each individual cell nucleus. The number of S-phase cells was unchanged after X-irradiation with 100 R, and slightly decreased after incubation with 0.1 μg per ml PbCl_2 . When both treatments were combined a strong decrease of S-phase cells was observed (to about 40%).

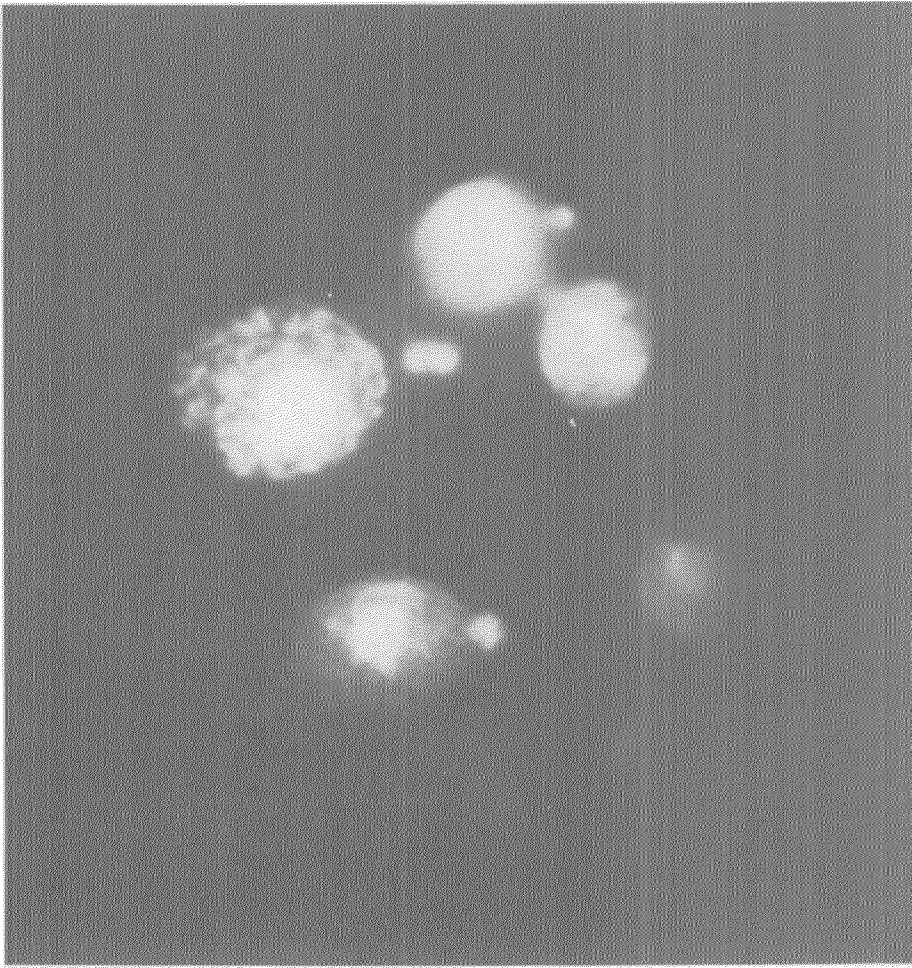


FIG.7. Isolated cell nuclei with micronuclei from pre-implanted mouse embryos. Some micronuclei are apparently just released from a nucleus. The chromatin is stained with ethidium bromide and shows fluorescence.

Under these conditions 30% of the cells appeared with a hyperploidic DNA content (higher than 4 n). After X-irradiation alone only 1% of the cells, and after lead incubation alone only 12% of the cells showed such a DNA content. These data demonstrate that the combined treatment has very severe consequences for the proliferation kinetics in these embryos. On the other hand, when the embryo were incubated with 1.0 $\mu\text{g PbCl}_2$ per ml medium the number of hyperploidic cells was less but the number of hypoploidic cells (DNA content smaller than 2 n) was increased. These changes may be caused by the formation of so called 'micronuclei', which will be explained below.

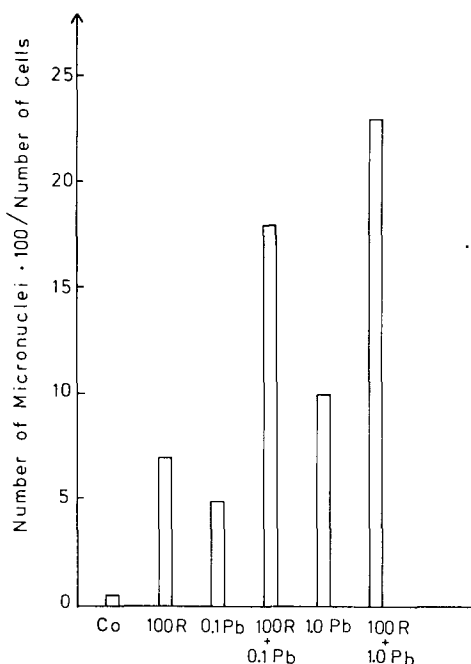


FIG. 8. The formation of micronuclei (percentage number of micronuclei per number of cells) in pre-implanted mouse embryos 72 hours p.c. (40 hours after in vitro culture). Untreated controls (Co); X-irradiation with 100 R (100 R); incubation with 0.1 μg PbCl_2 per ml culture medium (0.1 Pb); incubation with 1.0 μg PbCl_2 per ml culture medium (1.0 Pb) and the combined treatments (100 R + 0.1 Pb and 100 R + 1.0 Pb).

The cell nuclei of untreated embryos appear under the microscope in a very well formed, round shape (Fig. 3). However, cell nuclei from treated embryos especially after combined treatment look quite different. The nuclei show damaged areas like craters in these cases, and furthermore there appear smaller pieces of chromatin with a DNA content which is smaller than the haploid genome (Fig. 7). These bits of chromatin are called micronuclei [17]. This chromatin has a concise structure and appears as small nuclei.

The number of micronuclei is counted in relation to the number of cells. In untreated embryos the occurrence was 4 micronuclei per 1000 cells. After irradiation or incubation with lead a dramatic increase occurs (Fig. 8). A good dose effect relationship was observed with X-irradiation alone (unpublished results). A radiation dose of 25 R gave a significant effect. When the concentration of PbCl_2 was increased the number of micronuclei was also enhanced, but there was no proportionality (Fig. 8). However, the combination of PbCl_2 and X-irradiation produced a considerable rise in this effect. The combined effect was much higher than additive in comparison with the single treatments.

It is assumed that the occurrence of micronuclei is due to chromosomal damage as it is, for instance, observed with cytogenetic methods. It has been shown that lead itself can induce chromosomal breaks in human lymphocytes [18]. What kind of biological significance the appearance of micronuclei has must be evaluated. From the observation on the proliferation kinetics, it appears that 2–3 cell cycles after X-irradiation or incubation with lead so much chromosomal damage has been accumulated that parts of the chromatin are released and break off from the nuclei. From our observations we conclude that this happens during the interphase of the cell cycle or micronuclei are released from hyperploidic cell nuclei. Such events would also explain the appearance of hypoploidic nuclei after loss of larger amounts of chromatin.

COMBINED EFFECTS OF HEAT AND IRRADIATION

When mammalian cells and tissues are heated to 41°–45°C, a strong radiosensitizing effect is observed [19, 20]. Such an effect was also found when pre-implanted mouse embryos were incubated at 39°C instead of 37°C after X-irradiation with 200 R. When the incubation temperature was lowered to 37°C, 3 hours later only 20% of the embryos developed to blastocysts and hatched (200 R alone, 56%); 24 h incubation at 39°C after irradiation inhibited the hatching almost completely.

The investigation of the proliferation kinetics showed that X-irradiation combined with an elevated incubation temperature had a very strong effect on various parameters (Table I). The number of cell nuclei per embryo, the labelling index and the number of cells with S-phase DNA content decreased more than additive in comparison with the single treatments. Also the increase of hyperploidic cells was higher than additive (Table I). Some of these hyperploidic cell nuclei synthesize DNA as shown by autoradiography after incubation with ³H-thymidine.

In addition to mention in studies with lead, a large number of micronuclei were also seen in these experiments. This effect was strongest after the combined treatment again. The formation of micronuclei may also explain in part the observation that the labelling index was smaller than the number of cells with S-phase DNA content especially after combined treatment. Hyperploidic or G₂-phase cells lose DNA and show a S-phase DNA content, although they are not synthesizing DNA.

These experiments demonstrate that the embryo development is very sensitive to increase in temperature. The rise by 2 deg C to 39°C is not unrealistic, for instance with patients during medical treatment, and may be important for risk estimations for the embryo when X-ray exposure takes place during fever in the mother. The studies further demonstrate that the system may also be used to investigate combined effects between radiation and other agents besides substances.

TABLE I. DATA ON THE PROLIFERATION CHARACTERISTICS OF PRE-IMPLANTED MOUSE EMBRYOS CULTURED IN VITRO AND MEASURED AT THE MORULA STAGE (77 h p.c.; 44 h INCUBATION IN VITRO) *The number of embryos are given in brackets. The irradiation was performed 33 h p.c. The incubation at 39°C was begun directly after irradiation*

	37°C	200 R	39°C	39°C + 200 R
Number of nuclei/E	21 (27)	15 (44)	17 (43)	9 (20)
Labelling index (%)	66 (27)	45 (44)	74 (43)	22 (20)
Mitotic index (%)	7.06 (27)	3.4 (44)	6.1 (43)	1.2 (20)
S-phase nuclei (%)	70 (17)	70 (28)	70 (18)	43 (20)
DNA content				
Hyperploidic nuclei (%)	2 (17)	18 (28)	12 (18)	38 (20)

It can be concluded:

- (1) The in vitro culture of pre-implanted mouse embryos is a useful and sensitive model system for the study of combined effects by radiation and other agents.
- (2) Apart from the embryo development various parameters of cell proliferation can be measured.
- (3) The occurrence of micronuclei allows the possibility of observing cytogenetic damage to mammalian cells after combined treatment.
- (4) The system is relatively easy to work with and close to physiological conditions. It gives the opportunity to investigate interaction mechanism in a comparatively simple way.

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DISCUSSION

H. ALTMANN: Did you determine the DNA content per cell by ethidium bromide staining alone, and did you correct for the fact that in irradiated cells you have less intercalation in DNA?

C. STREFFER: We also measured the DNA content with acriflavine and checked our methods regularly with diploid lymphocytes. In our measurements we did not find that the chromatin showed less fluorescence after ethidium bromide staining after low radiation doses.

J.R. MAISIN: What is the radiation dose to the embryos from 200 $\mu\text{Ci/ml}$ of tritiated water?

C. STREFFER: About 240 rads under our conditions.

J.R. MAISIN: What percentage of normal embryos cultivated in vitro will develop in vivo?

C. STREFFER: About 30% of the cultured blastocysts develop in vivo after transplantation. After irradiation the percentage is lower, although only those blastocysts are transplanted which look intact under the microscope.

K.H. CHADWICK: You said that lead caused chromosome damage. What does it do to the DNA, and also what does actinomycin D do to DNA?

C. STREFFER: We do not know whether or in what amount lead reaches the cell nucleus or the DNA. We do, however, observe the formation of micronuclei which is an expression of chromosomal damage. Actinomycin D is apparently interchelating with DNA and it seems that these changes in structure enhance radiation damage. In addition, recovery from radiation damage is impaired by actinomycin D and it also radiosensitizes RNA synthesis, as can be demonstrated for instance by measuring the induction of tryptophane pyrrolase.

RADIATION-INDUCED DEVELOPMENTAL ANOMALIES IN MAMMALIAN EMBRYOS BY LOW DOSES AND INTERACTION WITH DRUGS, STRESS AND GENETIC FACTORS

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Abstract

RADIATION-INDUCED DEVELOPMENTAL ANOMALIES IN MAMMALIAN EMBRYOS BY LOW DOSES AND INTERACTION WITH DRUGS, STRESS AND GENETIC FACTORS.

The effect of low doses of radiation with different LET (140 kV X-rays, negative pions and 15 MeV electrons), as well as the interaction with drugs, genetic and stress factors, has been studied in rat and mouse embryos. Pregnant mice of two different strains (F/A and NMRI) and rats (Sprague-Dawley) were irradiated at day 8 or 9 of gestation. Four to five days after irradiation (with and without additional treatment) the foetuses were observed macro- and microscopically for developmental anomalies such as post-implantation loss, growth retardation, eye defects, exencephaly, cleft palate and limb defects. In both mice strains it was found that a radiation dose as low as 1 rad results in a significant increase in the rates of abnormal foetuses. Irradiation with peak pions (high LET) was more effective than 140 kV X-rays or 15 MeV electrons (RBE 1.4). Application of iodoacetamide and tetracyclines (Reverin, Ledermycin) before irradiation with X-rays led to a significant sensitization of radiation effects. The most impressive synergistic effect was shown with lucanthone (Miracil D) where the radiation damage after 50 rads was multiplied almost fourfold. With smaller radiation doses the injection of lucanthone led to various degrees of sensitization depending on both the mouse strain (genetic factors) and dosage used. Besides chemical substances, a short time restraint of pregnant females represents a stress situation which was teratogenic in mice, and may enhance radiation and chemically induced developmental anomalies. Combinations of modifying factors with different radiation might deserve further attention.

INTRODUCTION

The high radiosensitivity of developing organisms, which has been described in various mammalian species, is dependent on properties peculiar to the early stages of life [1]. In this connection the differentiation processes in embryonic cells are one of the most important biological parameters for radiation damage [2].

Experimental data on the effects of low radiation doses on morphological or functional development are limited. To our knowledge since 1965, when Ohzu [3] and Jacobsen [4] reported the teratogenic action after irradiation with 5 rads, no further report of investigation at this low dose level has been published.

In recent experiments using high LET radiation such as neutrons and helium ions the dose range varied between 25 and 1200 rads. The RBE values for embryo-lethal or teratogenic effects were between 1.5 and 4.5 with fission neutrons [5–7] and between 1.1 and 1.7 with helium ions [8], depending on the developmental stage at exposure. In the course of determining radiobiological qualities of negative pions, our experiments with the embryonic system showed a RBE factor of 1.3 in the plateau and 1.7 in the peak region compared with X-rays [9].

Earlier investigations using chemical substances that interfere with the cellular energy metabolism (iodoacetamide, tetracyclines) showed an enhancing effect on radiation-induced developmental damages in the rat and mouse [10–12]. Also lucanthone (Miracil D), an antischistosomal agent, proved to be an effective radiosensitizer [13]. We are now studying the interactions of drugs of general interest and other factors with radiation of different LET. The purpose of this report is the further evaluation of effects of low-dose irradiation with pions, electrons and X-rays on mouse embryos during organogenesis, and to summarize briefly some effects of radiation-modifying factors.

METHODS

Randomly bred albino mice of the NMRI and Füllinsdorf-Albino strain (F/A) were mated overnight and the pregnant females irradiated on day 8 of gestation. The animals were maintained in temperature-regulated rooms at $22^{\circ} \pm 1^{\circ}\text{C}$ with a 12-h light-dark cycle and were not anaesthetized during irradiation. For irradiation or sham-irradiation the females were confined individually in a well-ventilated, narrow plexiglass chamber. The 590 MeV-proton accelerator of the S.I.N. (Schweiz. Institut für Nuklearforschung, Villigen) was the source of negative pions, providing in the peak a dose rate of 1–2 rads/min. The dose was measured with a tissue-equivalent ionization chamber (EG & G). Fifteen MeV electrons were generated by the betatron at our institute with a dose rate of 0.9 rads/min. Irradiation with X-rays was performed at 140 kV; 6 mA; 1.0 mm Al + 0.25 mm Cu + 0.4 mm Sn; HVL, 0.96 mm Cu; 92.8 cm target-to-subject (embryo) distance; 0.7 rads/min. The absorbed dose in the approximate position of the embryos was measured with a Simplex-Universal dose meter for X-rays and with a Baldwin Farmer chamber for electrons.

The chemicals, freshly dissolved in sterile water or saline, were given ip 1/2 h or 1 h before irradiation. In order to obtain a complete and original

MACROSCOPIC MALFORMATIONS

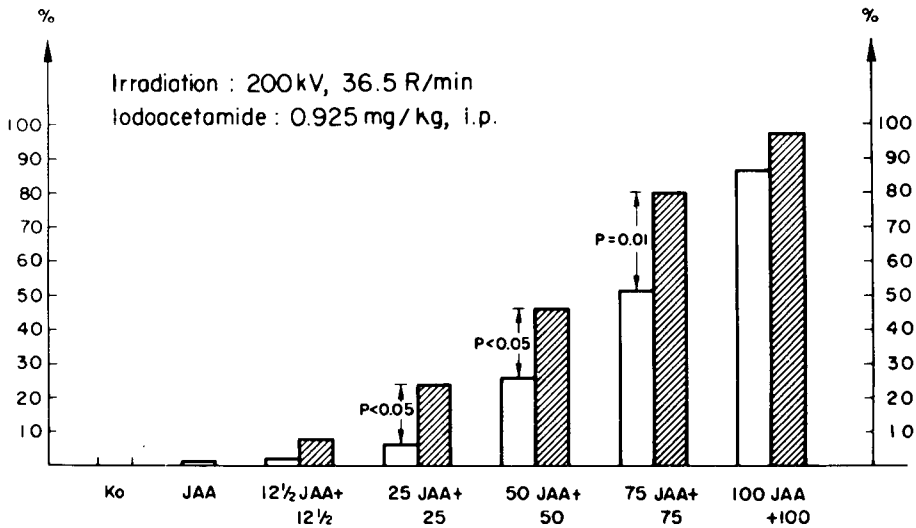


FIG.1. Externally visible abnormalities in rat fetuses at day 13 with and without injection of iodoacetamide 1 h before partial-body irradiation on day 9 of gestation.

picture of the extent of radiation damages relatively soon after treatment, i.e. on day 13 of gestation, the fetuses were removed and carefully examined.

The following developmental anomalies were tested: post-implantation resorption, growth reduction, eye and brain anomalies, and tail and limb defects. Further details concerning material and methods have been published elsewhere [14, 15].

RESULTS

In our initial work we tested the influence of iodoacetamide, a sulphydryl poison [16] and inhibitor of the ATP synthesis [17], on radiation damage in rat embryos. As Fig.1 shows, iodoacetamide itself was not teratogenic at the concentration used; however, the number of developmental anomalies in rat fetuses was increased when the chemical was injected 1 h before acute irradiation on day 9 of gestation. Sensitization could be observed within the whole dose range tested, although the degree was small with the dose of 100 rads, which by itself was highly teratogenic. In some combined treatments, i.e. iodoacetamide and 75 or 100 rads, the severity of eye anomalies was increased as more fetuses were

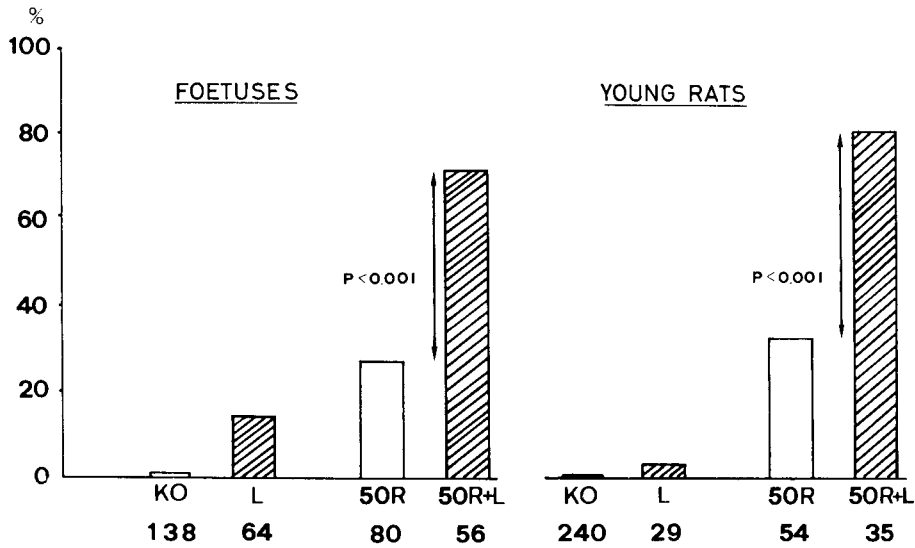


FIG.2. Eye anomalies (anophthalmia and microphthalmia) following injection of 160 mg/kg Ledermycin (L) and irradiation with 50 rads. Ledermycin application 1 h before irradiation on day 9 of gestation. Numbers of tested foetuses are given below the columns. Left: Anomalies of eyes in day 13 foetuses. Right: Abnormality rate in neonatal rats, 25 days p.p.

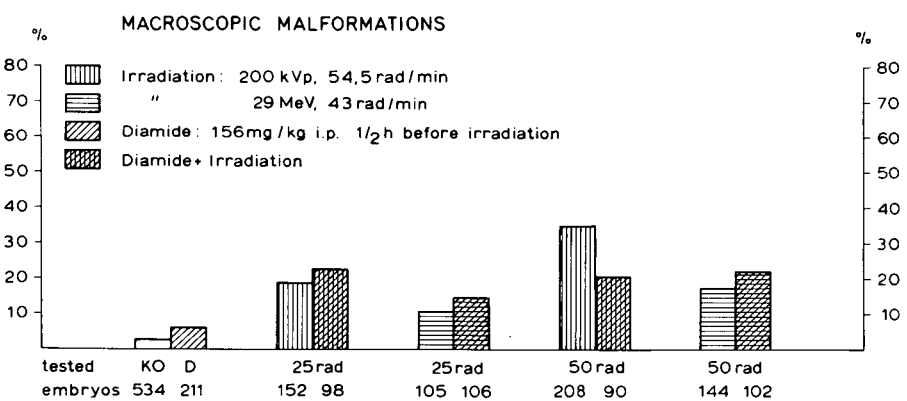


FIG.3. Incidence of malformations following irradiation with 200 kV X-rays and high energy photons with and without Diamide. Macroscopic examination on day 13 of gestation, i.e. 4 days after treatment.

affected with anophthalmia than after irradiation alone. Incidentally, this statement was confirmed by the evaluation of serial sections through the eye region at day 13 of gestation.

Similar enhanced effects under the same experimental conditions could be demonstrated also with two kinds of tetracyclines, namely Reverin and Ledermycin. If Reverin (Pyrrolidinomethyl-tetracycline) was injected (91 mg/kg; 1 h before irradiation with 50 rads) the number of eye defects increased from about 25% (irradiation only) to 58%. This increase was apparent not only at the prenatal but also at the postnatal examination, an observation which was made already in the experiments with iodoacetamide. As opposed to Reverin, Ledermycin (Demethyl-chloro-tetracycline) caused some toxic side effects on the pregnant animals. Yet the combined application of Ledermycin (160 mg/kg) and 50 rads revealed a significantly higher malformation rate than that of radiation alone (Fig.2). The figure shows that the combined effect of the two agents is more than a strict addition. It is of particular interest that in parallel experiments the numbers of anophthalmia in young rats at weaning (25 days after birth) exceeded those in foetuses examined at day 13 by a factor of seven.

When we tested Diamide (diazene dicarboxylic acid bis (N,N-di-methylamide)) as a potential sensitizer for embryonic damage in mice [18], no sensitizing effect could be found with radiation doses of 25 and 50 rads (Fig.3).

Continuing our studies we then chose lucanthone (1-diethyl-aminoethylamino-4-methyl-10-thioxanthenone), a substance reported to be an effective radiosensitizer for chromosome loss in *Drosophila* [19] and also increased radiation damage in HeLa cells [20] and mice [21].

The effects of lucanthone and irradiation with 13.5 rads on mouse embryos at day 8 of gestation are summarized in Fig.4. In both strains of mice a restraint of 20 min in the irradiation chamber resulted in a significant increase in the rate of developmental abnormalities compared with non-restrained foetuses. Only in NMRI foetuses the injection of lucanthone (70 mg/kg, ip) led to a remarkable reaction including the induction of growth retardation, eye defects and exencephalia. Interestingly the frequency of these effects was not further enhanced by restraining; this finding is in contrast to that on the F/A strain, where a four-fold increase in the number of abnormal foetuses can be seen after lucanthone and restraining. The irradiation with 13.5 rads X-rays revealed practically the same response in both strains without showing a significant increase in the abnormality rates compared with the restraint groups. Pretreatment of pregnant females with lucanthone 30 min before irradiation with X-rays resulted in high abnormality rates of 72% and 60% in F/A and NMRI foetuses respectively, which is significantly higher than after each single treatment alone. The effect is synergistic in F/A mice, and seems to be additive in the other strain.

Compared with X-rays the teratogenic effectiveness of negative pions is raised by a factor of 1.7 to 1.8. However, this greater efficiency of pions as well

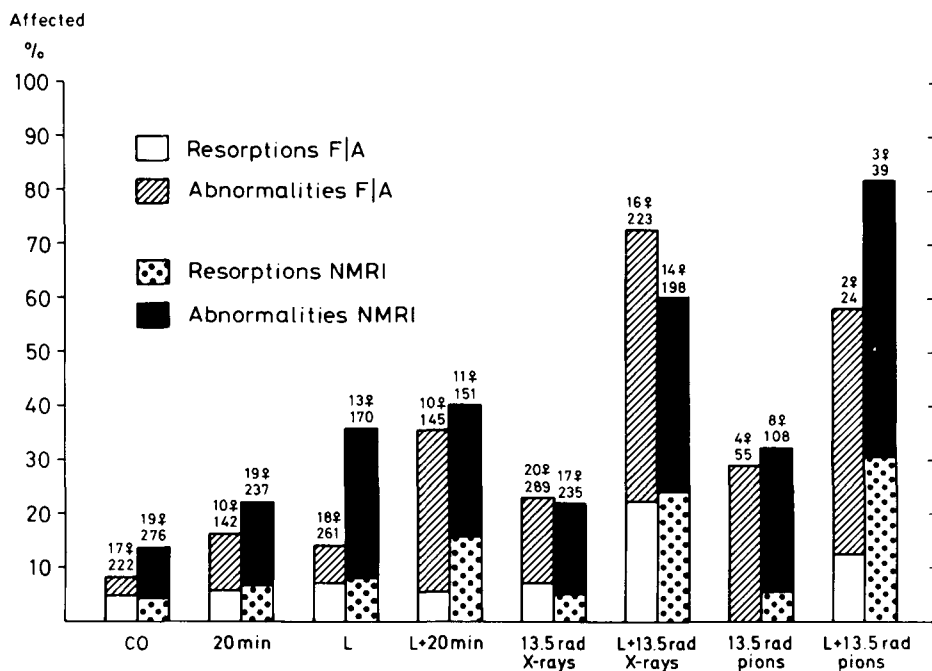


FIG.4. Incidence of resorptions and abnormalities in Füllinsdorf Albino (F/A) and NMRI foetuses on day 13 of gestation. Injection of lucanthone (L: 70 mg/kg) 30 min before irradiation with 13.5 rads on day 8 (dose rate: 0.7 rad/min). The number of pregnant females and implantations is shown above each column.

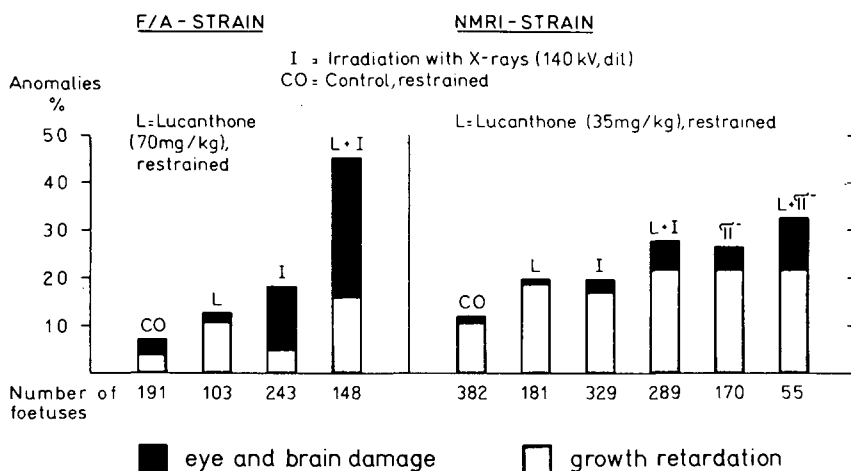


FIG.5. Developmental abnormalities in day 13 mouse foetuses of two different strains. Irradiation with 1 rad of protracted X-rays or peak pions (dose rate: 0.7 rad/min).

as the surprisingly high percentage of abnormal foetuses in the lucanthone + 13.5 rads pion group (NMRI-strain) must be verified by further experimental data. It is noteworthy that in this experiment some strain differences were found with regard to the incidence and type of malformations. We were quite surprised to find a relatively high number of exencephalia in the NMRI strain (up to 8%) but not in the F/A strain.

Finally, a report will be made on the effects of 1 rad on mouse embryos during the highly sensitive phase of the early organogenesis (day 8 of gestation). In this experiment the restraining of the females in the irradiation chamber for 1 min and 50 s (time required for exposure to 1 rad) again resulted in an increase of the abnormality rates; however, in contrast to the 20 min groups the differences compared with the non-restrained controls are statistically not significant. In the NMRI animals the dose of lucanthone has been reduced to 35 mg/kg, but the drug was still more effective than the dose of 70 mg/kg in the F/A mice (Fig.5).

The most striking finding in this preliminary study was that irradiation with only 1 rad X-rays, and especially with 1 rad pions, resulted in a significantly higher incidence of abnormal foetuses compared with restrained controls (Mann-Whitney U-tests, $p < 0.05$).

The injection of lucanthone 30 min before irradiation led to higher rates of abnormal foetuses in all three experimental series, but a synergistic effect is present only with the larger dose of lucanthone (F/A strain). The lack of a statistical difference in the NMRI strain suggests that the sensitizing effect of lucanthone may be dependent on the concentration used. So with half the dose (35 mg/kg) the effect in the combined treatment is merely additive. Combined with 1 rad pions the drug demonstrated a somewhat higher enhancing effect, inasmuch as the rate of post-implantation death and abnormalities amounted to 41%, which is 10% more than an addition of the two agents.

Qualitatively the effects consisted mainly of growth retardation indicated by smaller body size and a significant decrease in foetal weight, and, secondly, microphthalmia.

Microphthalmia was defined if the eye diameters were at least two standard deviations below the average of control values. Clearly, the two strains have dissimilar properties in radiation responses, namely a more frequent occurrence of growth retardation in NMRI mice. As in the experiments with 13.5 rads, exencephalia prevails in NMRI foetuses with frequencies of 3.50% (lucanthone alone) and 2.43% in the combined treatment. So far our results indicate that this severe brain damage may be produced rather by lucanthone than irradiation. In untreated foetuses exencephalia is a very rare event (2 cases in more than 1500 control foetuses).

In most recent and extended data on the effect of 1 rad on NMRI embryos we confirmed the earlier observations [14]. The incidence of post-implantation loss in the irradiated groups (as percentage of total implantations) is not

TABLE I. EFFECT OF PROTRACTED IRRADIATION WITH 1 rad ON DAY 8 OF GESTATION IN NMRI MICE
Macroscopical examination on day 13

	Number of pregnant mice	Number of foetuses examined	Number of resorptions	Foetuses		
				Retarded	Malformed	Normal
Control	64	841	46 5.19%	67 7.97%	15 1.79%	759 90.24%
Control restrained (1 min 50 s)	46	558	41 6.84%	63 11.29%	9 1.62%	486 87.09%
1 rad electrons (15 MeV)	25	307	32 ^a 9.44%	51 16.61%	9 2.93%	247 ^b 80.46%
1 rad X-rays (140 kV)	52	646	57 ^a 8.11%	112 17.34%	19 2.94%	515 ^b 79.72%
1 rad pions (peak)	22	283	26 ^a 8.41%	64 22.61%	14 4.94%	205 ^c 72.45%

^a Not different from restrained control ($p > 0.05$).

^b Significantly different from restrained control ($p < 0.05$).

^c Significantly different from restrained control ($p < 0.01$).

significantly increased compared with the restrained control (Table I). There is also no statistical difference in the extent or degree of abnormal foetuses in the three kinds of radiations tested. However, a trend is indicated towards decreasing effectiveness in the following order: pions > X-rays > electrons, which coincides with the corresponding decrease in the ionization densities. The RBE for producing abnormal foetuses is about 1.4 compared with X-rays or electrons. This is within the range of the values obtained in the 13.5 rads experiments.

DISCUSSION

The principal finding of this study is that low-level exposure to 1 rad of X-rays, electrons and negative pions has a significant deleterious influence on embryonic development in mice. Pronounced differences between irradiated and non-irradiated but restrained control foetuses were observed as regards the frequency of both eye anomalies and growth retardation. Concerning the latter criterion, two tentative conclusions may be drawn from the present data. Firstly, there is a clear association between growth retardation and teratogenesis since all grossly malformed foetuses (for example exencephaly) were also reduced in weight and body size. Secondly, the results indicate that growth retardation may be a more sensitive parameter of embryonic radiation damage than is teratogenesis, a concept which needs further investigations. At present we are investigating whether the impairment of growth can be restored in the course of the last six days of gestation or in further postnatal development. The relatively high percentage of abnormal foetuses in the experiments with 1 rad is difficult to explain. A provisional interpretation is the assumption that instead of cell killing the set damage is only sublethal. The cells remain viable but the capacity to form a normal structure or organism is impaired.

The results presented in this study show that stress situations caused by restraining the pregnant animals in irradiation chambers increased the spontaneous as well as experimentally induced abnormality rates. That a prolonged restraint (24 to 48 h) is teratogenic has been demonstrated in A/J mice [22], however, it is surprising that already 10 to 20 min are significantly effective in producing developmental anomalies [23].

This so-called 'cage-effect' was never observed in our earlier experiments using concentrated 200 kV X-ray irradiation, and is now under further investigation.

Another interesting fact is the finding that the embryonic radiation damage can be modified by application of different chemical substances (review in Ref. [24]). The possible mechanisms of the action of iodoacetamide, tetracyclines and lucanthone are discussed in our earlier publications [10–13]. Although these drugs may act at various sites in the cell, we assume that their

radiosensitizing effect is possibly related to an impairment of the energy metabolism. This hypothesis may be supported by the finding that iodoacetamide and tetracyclines reduced the membrane-dependent ATP production by depression of the oxidative phosphorylation in rat mitochondria [17] (unpublished data). This reduction of the energy metabolism may then lead to inhibition of the cellular repair capacity. Using similar experimental conditions but caffeine and chloroquine as sensitizer, Yielding and co-workers [25] also postulated the concept of repair inhibition. With regard to iodoacetamide, basic information relating to mechanisms and molecular aspects of sensitization is reviewed in detail by Quintiliani [26].

From the practical aspect lucanthone is of more interest since it is applied in clinical radiation therapy [27]. Chemically lucanthone resembles actinomycin D and also inhibits nucleic acid synthesis in different systems (see Ref.[13]). The mechanism of action of the drug on embryonic development as well as its radiosensitizing effect are not yet known. We plan to examine whether lucanthone may be acting in a way related to the drugs mentioned above.

As pointed out by Streffer [28], the possibility that radiosensitizing drugs may enhance the effects of even small radiation doses deserves further examination. In this respect the sensitizing effect of lucanthone with the dose of one rad is important, particularly concerning problems of radiation protection and health risks.

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DISCUSSION

M. KALIŠNIK: You have pointed out an important aspect to which attention should be paid in irradiation experiments, namely that the confinement of animals can provoke a severe stress, which could modify the effects of the irradiation itself. Your observation is consistent with our finding that a tremendous involution of the thymus occurred in mice confined in small compartments in plastic wheels for one hour daily. May I ask what were the exact dimensions of the chamber in which you kept the animals during irradiation or simulated irradiation?

C. MICHEL: As the field size in the case of pion irradiation was approximately $3 \times 5 \text{ cm}^2$ (85% isodose curve), a plexiglass phantom measuring $3 \times 3 \times 5 \text{ cm}$ was constructed.

C. STREFFER (*Chairman*): You showed teratogenic effects even with only one rad protracted X-irradiation. What does protraction mean in dose rate? Hitherto, you and other authors have been able to observe such effects (i.e. visible malformations) only after much higher radiation doses. Do you have any explanation for the higher radiosensitivity you have reported today? Also can you account for your finding that lucanthone was less effective in the higher dose (70 mg/kg)?

C. MICHEL: With protracted X-irradiation at 140 kV we have a dose rate of 0.7 rad/min, which is within the range of that with negative pions in the peak. We used a radiation dose of 1 rad for the first time in this study. In our earlier experiments the lowest teratogenic dose was 12.5 rads, applied to rat embryos at day 9 of gestation. Since we are working with outbred strains of mice, the genetically heterogeneous animals may show stronger reactions. Different radiosensitivities may therefore arise due to different genetical backgrounds.

I believe that it is also important to study animals, whose resistance is not limited by a possible narrow reaction norm.

With regard to the effects of lucanthone, the two strains of mice reacted in a very different way both quantitatively and qualitatively (see Ref. [14]). Thus in NMRI embryos the lower dose (35 mg/kg) resulted in a higher frequency of anomalies than 70 mg/kg in F/A embryos.

We assume that the higher susceptibility of NMRI embryos is due to their being at different developmental stages at the time of treatment.

INTERACTION OF CHEMICAL MUTAGENS AND RADIATION IN THE INDUCTION OF MALIGNANCY

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Abstract

INTERACTION OF CHEMICAL MUTAGENS AND RADIATION IN THE INDUCTION OF MALIGNANCY.

Using the molecular theory of radiation action a mechanism is derived which predicts that a synergistic interaction can arise between a chemical mutagen and radiation. The synergism is expected to arise from the interaction of single-strand lesions induced by the chemical mutagen and single-strand breaks induced by the radiation. The mathematical derivation leads to three conditions which must be satisfied for a coherent analysis. Data are analysed for the combination of diamide and radiation which satisfy these conditions, and the analysis is extended to the combination of BudR and radiation for chromosomal aberrations and survival. The theoretical analysis is developed further to the induction of cancer, and it is shown that the synergistic interaction between a chemical mutagen and radiation would be expected to lead to an increase in the incidence of cancer at low radiation doses. The implications of the model for radiological and environmental protection are discussed.

1. INTRODUCTION

The biology of cancer is highly complex, and many different physical and chemical factors have been found to affect its incidence. Some of these factors appear to interact with each other in influencing the incidence. At present, at least 80% of cancer is ascribed to environmental causes [1], and the identification and elimination of the environmental factors which cause these cancers is suggested as the most promising way to reduce the cancer problem. Since Ames and co-workers [2, 3] developed the mutagen screening test, and showed that there is a most convincing correlation between known carcinogens and mutagenic activity, it has become generally accepted that mutation plays an important role in the development of many, if not all, cancers. It seems likely, therefore, that several of the environmental factors will prove to be environmental mutagens.

In a previous publication [4] we have presented a theoretical approach to describe the induction of cancer by radiation which assumes that the radiation acts by inducing a somatic mutation and converts a potentially malignant cell into a

pre-malignant state. In this study we develop the theory further to demonstrate a mechanism whereby radiation and a chemical mutagen may synergistically interact to give an increased incidence of cancer, which is especially significant at low doses of radiation.

2. THEORETICAL BACKGROUND

In the development of the molecular theory of radiation action [5] we have assumed that radiation-induced DNA double-strand breaks are the most critical lesions which can lead to cell reproductive death, chromosomal aberrations, mutations and malignancy. We have also assumed that the mean number (N) of double-strand breaks induced per cell by a dose (D) of radiation is given by

$$N = \alpha_0 D + \beta_0 D^2 \quad (1)$$

where α_0 is the mean number of double-strand breaks per cell per unit dose induced in one radiation event

β_0 is the mean number of double-strand breaks per cell per unit dose squared which result from the combination of two independently induced single-strand breaks.

If p is the probability per double-strand break that a break leads to cell reproductive death, then cell survival (S) is given by

$$S = \exp - pN = \exp - p(\alpha_0 D + \beta_0 D^2) \quad (2)$$

Dugle and co-workers [6] have presented experimental evidence that supports the assumption of the linear-quadratic dose relationship for DNA double-strand breaks induced in cells, and also the association between double-strand breaks and cell reproductive death.

In direct analogy with the above argument we have also suggested that, if an agent interacts with cellular DNA to form single-strand lesions, then double-stranded lesions will arise through the combination of pairs of single-stranded lesions close to each other on opposite strands of the DNA. If X represents the exposure of the cells to the agent, then the mean number of single-strand lesions can be represented by $k''X$ and the mean number of combined pairs per cell can be represented by kX^2 . If the cytotoxic action of the agent arises from the double-strand lesions, then cell survival (S) following exposure (X) to the agent is given, in the first instance, by

$$S = \exp - pkX^2 \quad (3)$$

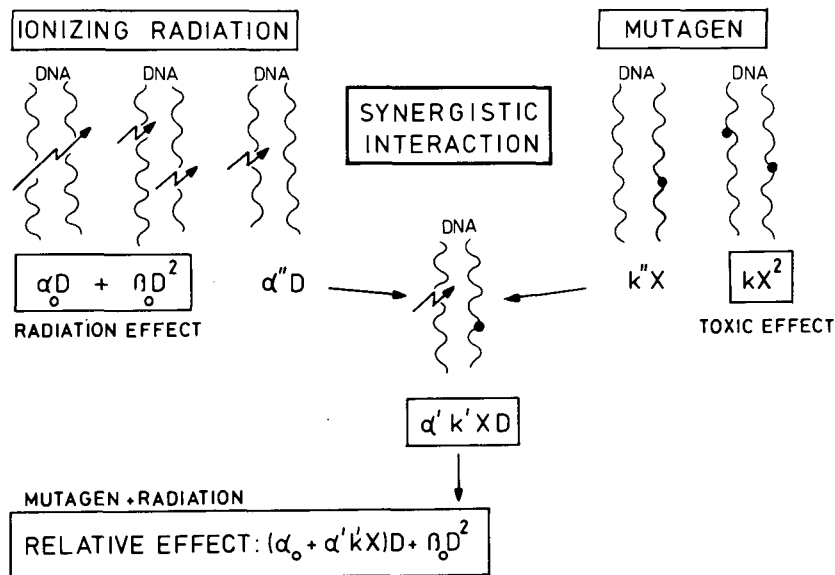


FIG.1. Schematic representation of the action of radiation and chemical mutagens on cells and the synergistic interaction of a combined treatment.

In addition to double-strand breaks, radiation also induces a large number of single-strand breaks which are normally perfectly repaired and do not contribute to the radiation effect. However, when radiation is combined with a chemical which induces single-strand lesions, then an additional number of double-strand lesions can arise from the interaction of a radiation-induced single-strand break and a chemically induced single-strand lesion. The schematic representation of the proposed synergistic interaction of radiation with a chemical mutagen is given in Fig.1. If $\alpha'' D$ is the mean number of single-strand breaks per cell induced by the radiation, then the additional mean number of double-strand lesions per cell which arise from the combined treatment is given by $\alpha' k' X D$, where α' and k' are related to α'' and k'' respectively.

Thus, following a combined radiation and chemical treatment the total number of double-strand lesions is

$$\alpha_0 D + \alpha' k' X D + \beta_0 D^2 + kX^2$$

and the survival can be written as

$$S = \exp - p [(\alpha_0 + \alpha' k' X) D + \beta_0 D^2 + kX^2] \quad (4)$$

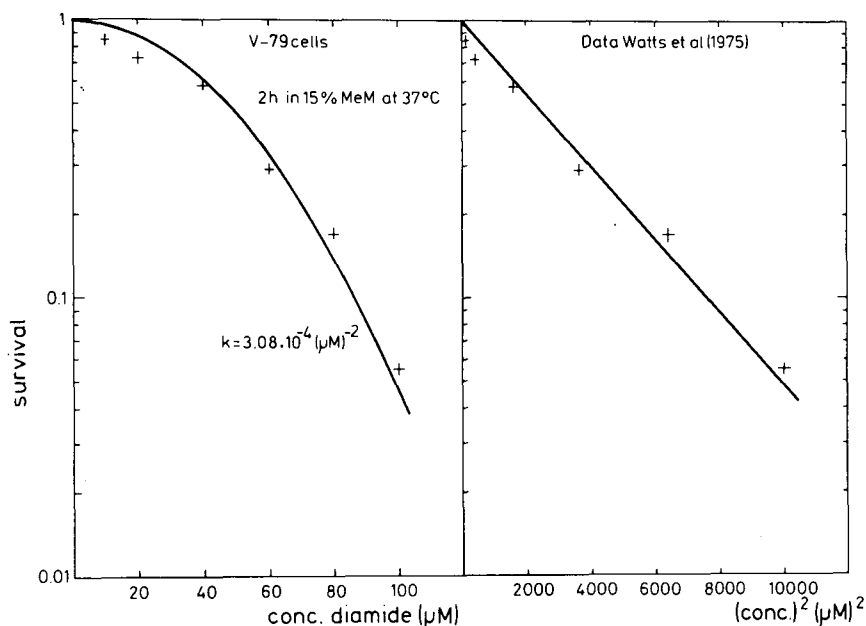


FIG.2. An analysis of the toxic action of the hypoxic cell sensitizer diamide on V-79 Chinese hamster cells [8] in accordance with Equation (3).

Following a chemical treatment at one level of exposure (constant X), the relative radiation dose-survival relationship is given by

$$S = \exp - p [(\alpha_0 + \alpha'k'X) D + \beta_0 D^2] \quad (5)$$

Thus, on the basis of the proposed mechanism, a comprehensive mathematical analysis of the synergistic interaction of a chemical with radiation is only possible if three conditions are satisfied:

- The chemical should attack DNA and its cytotoxic activity should be dependent on the square of the exposure of the cells to the chemical (Equation (3)).
- The radiation survival curves following combined treatments of a chemical with radiation should all fit linear-quadratic dose relationships which have the same $p\beta$ coefficient as the survival following radiation alone. The change in survival which results from the combined treatments is reflected in a change in the $p\alpha$ coefficient (Equation (5)).
- The increase in the $p\alpha$ coefficient for different combinations of chemical plus radiation should be linearly related to the exposure of the chemical.

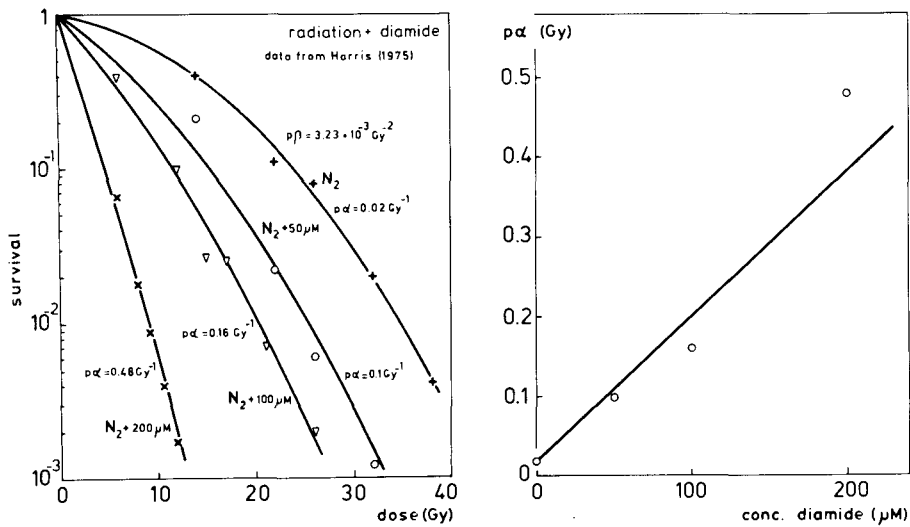


FIG.3. An analysis of the combined action of diamide and radiation on V-79 Chinese hamster cells [9] in accordance with Equation (5).

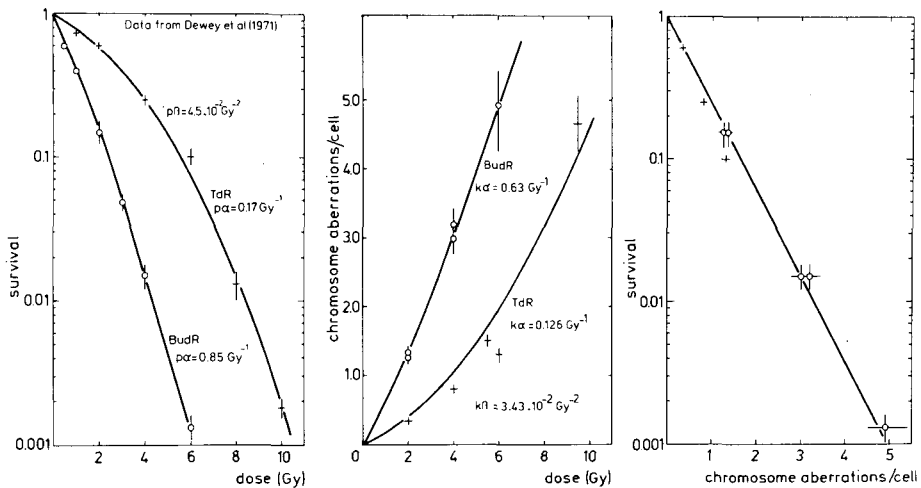


FIG.4. An analysis of the combined action of BudR and radiation on survival and chromosomal aberrations in V-79 Chinese hamster cells [13] and the correlation according to Equation (8).

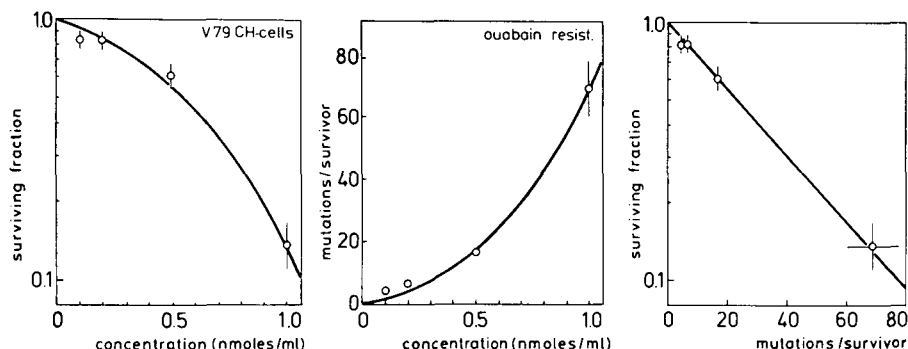


FIG.5. Cell survival and the induction of ouabain-resistant colonies following a treatment with the synisomer of benzo[a]pyrene 7,8-diol, 9,10-oxide in V-79 Chinese hamster cells [17] and the correlation according to Equation (11).

In some cases the survival curve following chemical treatment alone may not always be purely related to the square of the exposure but may also show a linear component [7]. However, as long as the chemical survival curve exhibits a significant quadratic component a synergistic interaction with radiation may be expected.

Figures 2 and 3 present an analysis of cell survival data for diamide and radiation which satisfy all three requirements for the analysis of a synergistic interaction as proposed in the theory. We have already published similar analyses for data with UV and radiation, CCNU and radiation [10] and a Pt complex and radiation [7].

The theory can also be extended to chromosomal aberrations when it is assumed that a DNA double-strand break may lead to an aberration [11, 12]. The yield (Y) of aberrations following a dose (D) of radiation can be written as

$$Y = K (\alpha_0 D + \beta_0 D^2) \quad (6)$$

where K is a constant which depends on the type of aberration and the scoring efficiency.

By analogy with cell survival, the yield of chromosomal aberrations following a chemical exposure is $Y = K (kX^2)$, and following a combined treatment of chemical and radiation the relative yield is given by

$$Y = K [(\alpha_0 + \alpha'k'X)D + \beta_0 D^2] \quad (7)$$

Figure 4 shows the analysis of cell survival and chromosomal aberration yield in cells treated with BudR and radiation. In both cases the β coefficient of all the curves remains constant, only the α coefficient changes by the same factor in

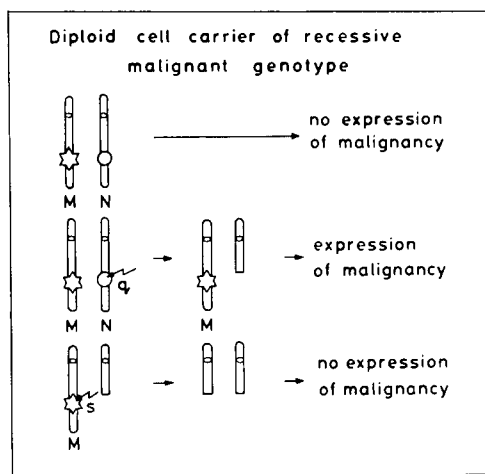


FIG.6. Schematic representation of how a radiation-induced aberration or mutation can convert a diploid cell which carries the recessive malignant genotype into a potentially cancerous state, and also how this state can be suppressed by radiation.

both biological end points. The third part of the figure demonstrates the direct correlation between survival and chromosomal aberrations which is theoretically predicted from Equations (2) and (6) or (5) and (7) to be

$$\ln s = -\frac{p}{K} Y \quad (8)$$

If q is the probability that a DNA double-strand break leads to a specific mutation, then the mutation frequency (M) per surviving cell is given by

$$M = [1 - \exp - q(\alpha_0 D + \beta_0 D^2)] \quad (9)$$

and, when M is small

$$M = q(\alpha_0 D + \beta_0 D^2) \quad (10)$$

The combination of Equations (2) and (10) leads to the prediction of a direct correlation between mutation and survival [14] which is given by

$$\ln S = -\frac{p}{q} M \quad (11)$$

Figure 5 presents an example of this correlation for a chemical treatment; examples of this correlation for radiation can be found in literature [5, 14–16].

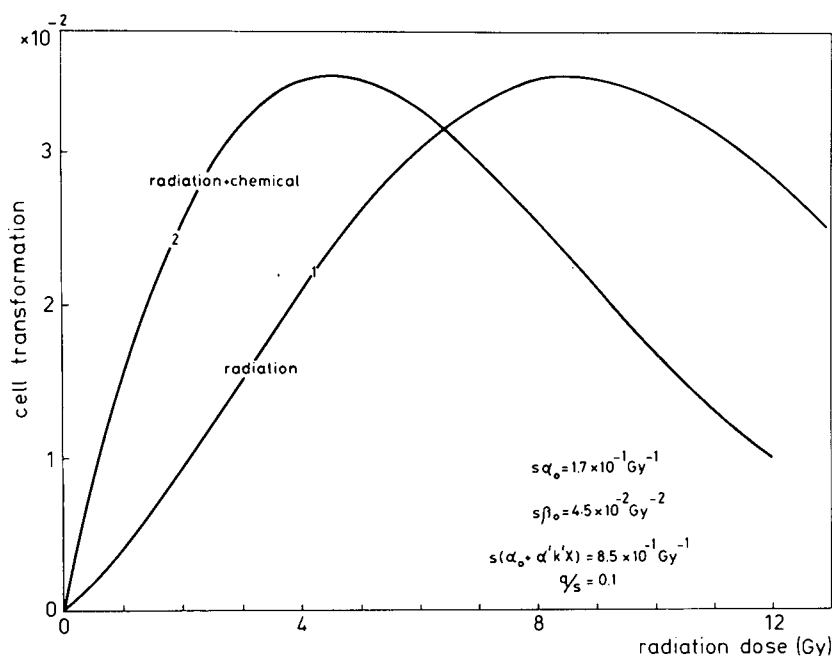


FIG. 7. A hypothetical representation of the induction of cell transformation for radiation, according to Equation (12), and the synergistic combination of radiation with a chemical mutagen, according to Equation (13). The figure can be compared with that presented by Borek and Hall [21] for cell transformation. Note that the influence of the chemical is revealed in an increase in the incidence at low doses. (The relative values of the parameters used in calculating these curves have been taken from Fig. 4.)

Previously, we have derived an equation to describe the induction of malignancy by radiation [4, 5] by assuming that the factor which controls the malignant nature of a cell behaves as a recessive genetic character [18–20]. Using this assumption it can be argued that in a cell which carries the malignant genotype, a mutation or aberration in the normal homologous controlling chromosome will make the cell pre-cancerous, but at the same time a mutation or aberration in the chromosome which carries the malignant genotype could suppress the expression of malignancy. This is illustrated schematically in Fig. 6 for a diploid cell. Thus the equation for the induction of malignancy per surviving cell by radiation contains two terms, one for the induction, one for the suppression, and is given by

$$T = \left\{ 1 - \exp[-q(\alpha_0 D + \beta_0 D^2)] \right\} \exp[-s(\alpha_0 D + \beta_0 D^2)] \quad (12)$$

By analogy with Equations (5) and (7) the equation for the induction of malignancy per surviving cell by a combination of chemical plus radiation can be written as

$$T = \left\{ 1 - \exp[-q((\alpha_0 + \alpha'k'X)D + \beta_0 D^2)] \right\} \\ \times \exp[-s((\alpha_0 + \alpha'k'X)D + \beta_0 D^2)] \quad (13)^*$$

Similar equations can be derived for the induction of malignancy per irradiated cell (as in animals) by adding an extra term describing cell survival, Equations (12) and (13) are modified so that $s \rightarrow (p + s)$.

Figure 7 presents Equations (12) and (13) drawn using the coefficients for $p\alpha$ and $p\beta$ derived for the data presented in Fig.4.

3. DISCUSSION

The analysis of the synergistic interaction between radiation and chemical mutagens presented here is only possible because in deriving the molecular theory of radiation action we have assumed that a specific molecular damage, the DNA double-strand break, is the critical radiation-induced lesion which leads to the different biological end points. The theory defines three different conditions which should be satisfied by the experimental data for the analysis of a synergistic interaction. The fact that these three conditions are satisfied for diamide, UV and CCNU [8] and a Pt complex [7] where synergism has been observed gives support to the initial basic assumptions of the molecular theory.

We prefer, at present, to remain vague about the exact nature of the double-strand lesions which result from chemical mutagen interaction with the DNA. In some cases double-strand breaks may result, but in other cases distortions or cross-links or base damage may lead to critical double-stranded lesions which do not form strand breaks. It is interesting to note in this respect that in the cases when strand breaks do not result, the relative values of the probabilities p , k , q and s may differ from those found for radiation. This does not alter the basic conclusions of this study, nor the validity of the correlation Equations (8) and (11), though it does imply that the slope of the straight lines, given by p/k and p/q , may differ for different treatments. Figure 4 indicates that for the combination of BudR and radiation the values of p and k remain the same as those for radiation alone.

* This equation is an approximation and only valid when kX^2 is small.

Two important conclusions result from this theoretical analysis; the first is that a synergistic interaction between radiation and any other agent which causes DNA single-strand damage can be expected. The second conclusion is that the synergistic interaction reveals itself in an increase in the proportion of the effect which is linearly related to radiation dose and which is therefore of prime importance for radiological protection. The theory predicts that the combination of a chemical mutagen and radiation will lead to an increased incidence of malignancy at low doses of radiation. It is possible to extend the arguments presented in this study further to consider the possible synergistic interaction of two different chemical mutagens; from the point of view of environmental hazard this sort of effect could be far more important and more insidious.

One problem which we have not considered here is that of cellular repair; we know that radiation-induced single-strand breaks are rapidly repaired in normal mammalian cells, and this implies that the timing between experimental treatments can be critical. We know far less about the repair of chemically induced DNA damage. This question of repair could have important consequences for the determination of incidence for the simultaneous chronic exposure of cells to both chemicals and radiation.

The synergistic mechanism described here is only one way in which chemicals and radiation may interact to increase the incidence of malignancy; it is a typical interaction which can occur when both agents are by themselves carcinogenic. Another mode of interaction is that of promotion, when application of a chemical, which by itself has no carcinogenic action, may stimulate a radiation-transformed cell to divide and express its malignant nature. There are several examples of an enhanced incidence of malignancy arising from the combination of chemicals with radiation; for example, radiation with carbon tetrachloride [22], radiation with ethionine [23], radiation with benzo-a-pyrene [24] and the well-known effect on lung cancer found for uranium miners who smoked [25, 26]. Some of these cases are probably the result of a synergistic interaction between the radiation and a chemical mutagen, although the case of radiation with carbon tetrachloride is more probably a question of a 'promotor effect' rather than synergism. In our opinion, suitable epidemiological studies on the incidence of cancer from a combination of two agents could reveal other important effects of synergism both for the combination of radiation with other mutagenic agents and for the combination of two chemical mutagens.

In view of the fact that 80% of cancer is thought to be caused by environmental agents, many of which are most probably chemical mutagens, it seems likely that some form of integral control of all environmental mutagens will eventually be necessary. At the same time research into the combined interaction of different mutagenic agents and the mechanism which is involved at the cellular level would lead to a deeper insight into the true environmental problem of malignancy, and could form the basis from which an integrated control could be developed.

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DISCUSSION

H. ALTMANN: In your elegant model you have correlated only double-strand breaks with mutation and cancer, and you told us that single-strand breaks do not contribute to these effects because this DNA damage is repaired very quickly. However, especially in T-lymphocytes, we have high levels of terminal nucleotidyltransferases and, if a single-strand break is not rejoined immediately, an additional nucleotide can be incorporated and you may get point mutations. How can this fact be included in your model?

This mechanism may be one factor in the induction of leukaemia and lymphomas by very low doses of ionizing radiation.

H.P. LEENHOUTS: The eukaryotic cell has a very well-known system for repairing DNA single-strand breaks: all the information necessary for it is still on the opposite strand and, normally, cells show no radiation effect as a result of single-strand breaks. Point mutations are most likely to be the result of repair attempts of the cell following the induction of a DNA double-strand break, and this fits quite well with what we see in experimental data.

G.A. SACHER: Your model leaves me in serious perplexity on two points. First, the function $\alpha D + \beta D^2$, which you give for a double-strand break, is asserted to be proportional to the yield of visible chromosome aberrations. However, chromosome rearrangements require the occurrence of two breaks, usually on separate chromosomes. Lea and Catcheside, and others before and since, have shown that the probability of a two-break rearrangement is a quadratic function of the probability of a single break. Hence, your theory seems to be at odds with a large body of cytogenetic evidence.

Second, the ratio of α to β for single-strand breaks is said to be the same as the α/β ratio for the production of visible chromosome abnormalities, yet the geometrical factors are very different, as double-strand breaks produced by two absorptions can be separated by only a small number of nucleotides. The result is that the α/β ratio must be very small compared with that for two independent chromosome breaks that are essentially equiprobable.

H.P. LEENHOUTS: We are aware that our model implies that scorable chromosomal aberrations, especially so-called two-break rearrangements, have to be formed on the basis of only one DNA double-strand break. In our opinion only the first break is induced by radiation: the second is induced enzymatically in the cell's attempt to repair the double-strand break. According to a model introduced by Resnick, this can lead either to restitution without formation of a scorable aberration, or to an exchange in two double-stranded DNA molecules, which at the chromosomal level is an exchange of chromatid arms (see Ref. [10]). Inclusion of this repair model in the molecular theory of radiation action leads to a linear-quadratic dose relationship for chromosomal aberrations, as seen experimentally. So all α/β ratios are connected to the production of DNA double-strand breaks.

The distances involved cannot easily be deduced as, apart from physical factors, chemical and biological (repair) factors are involved, which make calculation complicated.

A.M. STEWART: In theory, synergism could be due not to chemical carcinogens and radiation having similar (mutagenic) effects but to the chemicals damaging lymphocytes (or other components of the R.E.S.) and thus preventing the detection of any mutant cells created by the radiation. Likewise, the effects of cancer initiators and promoters could be due to the former being mutagens (or creators of self-replicating sources of foreign proteins) and the latter damaging the immune system and thus preventing recognition of mutant cell species.

Have you considered this alternative model for carcinogenesis?

H.P. LEENHOUTS: We know that other types of synergism may also play a role in carcinogenesis, especially those chemicals which 'promote' the radiation effect. We have not considered these other types, but hope that future experimental data will show which interaction model plays a role under various circumstances.

G.W. BARENSEN: On the basis of microdosimetric data it can be predicted that for your assumption of double-strand breaks in DNA to be equally frequent for the α and β terms, doses in excess of 100 000 rads are required, whereas actually the values of α/β for cell survival are in the range of 300–1500 rads. Thus there is a serious conflict between your hypothesis and microdosimetric data.

H.P. LEENHOUTS: When two models are in conflict, at least one of them is wrong, and not necessarily our radiation model. We are sure that microdosimetry fails to take proper account of chemical and biological factors, leading to the discrepancies you mentioned.

G.W. BARENSEN: I showed in my paper¹ that for X-rays and gamma-rays values of p_1/p_2 for chromosome aberrations are much smaller than the values of a_1/a_2 for cell reproductive death. Thus your hypothesis that these values are equal is in serious conflict with experimental data for many cell lines.

H.P. LEENHOUTS: When one determines cell survival and chromosomal aberrations in the same cell under the same experimental conditions, one finds a strict correlation between the logarithm of survival and the chromosomal aberration yield irrespective of the type of radiation, indicating the same ratio of the linear to the quadratic term for both radiation end points.

K.F. BAVERSTOCK: In view of the simplicity of the model you propose I should like to ask whether you have tested it over a wider range of conditions, for example for high LET radiation in conjunction with chemicals, and for radiation in conjunction with chemicals in the absence of oxygen. Also, do you predict synergism between two radiations of differing qualities but delivered simultaneously?

¹ BARENSEN, G.W., "Fundamental aspects of cancer induction in relation to the effectiveness of small doses of radiation", these Proceedings 2, IAEA-SM-224/401.

H.P. LEENHOUTS: We have not yet had an opportunity to analyse data on the simultaneous application of chemicals with high LET radiation, but the model is readily applicable for anoxic radiation conditions (see Ref. [8]). We do indeed predict synergism between two types of radiation; it is dependent on the magnitude of the β -terms of the radiations, when applied separately, and might be negligible when one of the radiations has no significant β -term.

ETUDE EXPERIMENTALE DE L'ACTION CO-CARCINOGENIQUE DU RADON-222 ET DE LA BENZO-5, 6 FLAVONE

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Abstract-Résumé

EXPERIMENTAL STUDY OF THE COCARCINOGENIC EFFECT OF RADON-222 AND BENZO-5, 6 FLAVONE.

Benzo-5,6 flavone is a chemical compound that is not known to be carcinogenic but which is a strong inducer of microsomal enzymes for the cells of pulmonary tissue. Several groups of animals received 6000 WLM of radon over two months. This dose produces lung cancers in over 50% of the animals with a latency time of more than 15 months after the start of treatment. The first group of 20 rats received 25 mg/kg of benzoflavone in weekly intraperitoneal injections for 13 weeks. The first injection was given three months after the end of the radon exposure. The first lung cancers appeared even before the end of the treatment and all the animals showed cancers within two months. All the tumours were of the epidermoid, multifocal type and invaded the lung. The animals that had been exposed to radon only did not show any lesions by this date. The second group of eight rats received the same benzoflavone treatment, but beginning one year later, i.e. 16 months after the start of the radon exposure. No differences were observed between the pulmonary lesions in these animals and those that had received radon only. The animals that were treated with benzoflavone only showed no pulmonary lesions. In parallel with the experiment on the cocarcinogenic effects of radon and benzoflavone, microsomal enzyme doses were given to other animals that had received the same radon dose but at different times. The results should help to explain the cocarcinogenic effect of benzoflavone.

ETUDE EXPERIMENTALE DE L'ACTION CO-CARCINOGENIQUE DU RADON-222 ET DE LA BENZO-5, 6 FLAVONE.

La benzo-5, 6 flavone est un composé chimique qui n'est pas connu comme étant un carcinogène, mais qui possède une action importante d'inducteur d'enzymes microsomaux

pour les cellules du tissu pulmonaire. Plusieurs groupes d'animaux ont reçu 6000 WLM de radon en deux mois. Cette dose entraîne l'apparition de cancers pulmonaires chez plus de 50% des animaux avec un temps de latence supérieur à 15 mois après le début de la contamination. Au premier groupe de 20 rats, on a administré par voie intrapéritonéale de la benzoflavone à raison de 25 mg/kg et d'une injection par semaine et ce pendant 13 semaines. La première injection a été pratiquée trois mois après la fin de l'inhalation de radon. Avant même la fin du traitement, les premiers cancers pulmonaires sont apparus et tous les animaux en ont présenté en moins de deux mois. Toutes ces tumeurs sont de type épidermoïde, multifocales et envahissent le poumon. A cette date, les animaux ayant reçu seulement du radon ne montrent aucune lésion pulmonaire. Avec le deuxième groupe de 8 rats, on a pratiqué le même traitement par la benzoflavone mais en commençant un an plus tard, soit 16 mois après le début du radon. Dans ce cas, on n'a pas observé de différence entre les lésions pulmonaires des animaux traités et de ceux qui n'ont reçu que du radon. Les animaux n'ayant reçu que la benzoflavone n'ont pas présenté de lésions pulmonaires. En parallèle avec l'expérience sur les effets co-carcinogènes du radon et de la benzoflavone, on a pratiqué sur d'autres animaux ayant reçu la même dose de radon, mais à des temps différents, des dosages d'enzymes microsomaux. Des résultats, on peut envisager une explication de l'effet co-carcinogène de la benzoflavone.

Des travaux expérimentaux ont mis en évidence la présence dans le tissu pulmonaire d'enzymes microsomiques. Ceux-ci jouent un rôle important dans l'action des substances étrangères. Comme il a été montré pour le foie, ces enzymes peuvent être induits ou inhibés dans différentes conditions. Wattenberg et al. [1-3] ont mis en évidence l'action inductrice au niveau des poumons des phénothiazines et des flavones. Nous avons comparé cette action inductrice chez des animaux témoins et chez des animaux préalablement soumis à des inhalations chroniques de radon et de ses produits de filiation à la dose de 6000 WLM. En parallèle avec ces études enzymatiques, un groupe d'animaux a été conservé pour étudier les effets continus à long terme du radon et de la benzoflavone (BF). Une telle procédure est systématique dans le laboratoire où sont toujours comparés effets précoces et effets à long terme.

D'autres groupes d'animaux ont été soumis au radon seul ou à la benzoflavone seule. Enfin, à titre de comparaison, nous avons aussi utilisé le méthylcholantrène dont les effets inducteurs sont bien connus. Nous avons aussi fait varier les intervalles de temps séparant la fin de l'inhalation du radon et le début du traitement à la benzonaphtoflavone ou au méthylcholantrène, ce qui faisait varier obligatoirement l'âge des animaux au moment du traitement. Pour nos expériences, nous avons utilisé des rats mâles de race Sprague Dawley SPF, âgés de 5 mois au début de l'inhalation de radon. Les inhalations de radon et les examens anatomo-pathologiques ont été pratiqués avec les mêmes techniques que celles décrites par Chameaud et al. [4].

La première série d'animaux était composée de 20 rats ayant inhalé du radon à la dose de 6000 WLM en 10 semaines.

Dix semaines après la fin de l'inhalation de radon, en même temps que des dosages d'enzymes étaient effectués sur d'autres rats du même lot, nous avons injecté en intrapéritonéale de la BF pendant 12 semaines à raison de une fois par semaine à la dose de 25 mg/kg.

Les rats sont morts à des temps variant de 88 à 144 jours après le premier jour d'injection de la BF. Nous avons trouvé dans les poumons de tous ces animaux des lésions multiples à tous les stades de malignité. Ces lésions étaient multifocales et comprenaient des papillomes inversés, des métaplasies et des carcinomes épidermoïdes kératinisés et très différenciés.

Nous avons dans cette série 100% de cancers pulmonaires.

Pour la deuxième série, nous avons utilisé 8 rats ayant reçu comme dans l'expérience précédente du radon pendant 10 semaines à 6000 WLM, mais nous commençons les injections intrapéritonéales de BF (avec la même posologie que pour la première série) 65 semaines après la fin de l'inhalation de radon.

Ces rats sont morts de 30 à 111 jours après la première injection de BF. Deux rats, morts à 30 et 48 jours, ne présentaient aucune lésion. Les autres rats présentaient la même gamme de lésions que dans la première série, mais nous avons trouvé également des carcinomes bronchiolo-alvéolaires à stroma cancérisé caractéristiques du radon.

La troisième série, qui se composait de 10 rats, a reçu uniquement de la BF, au même âge que les rats de la première série; les animaux ont été sacrifiés entre 80 et 360 jours après la première injection de BF (utilisée aux mêmes doses).

Nous avons trouvé dans le poumon d'un seul animal, sacrifié après 107 jours, un carcinome épidermoïde isolé.

Chez tous les autres rats les poumons ainsi que le reste de l'organisme étaient parfaitement sains, sans lésions anatomo-pathologiques apparentes.

Les 8 rats de la quatrième série ont reçu aussi de la BF pure, mais à un âge correspondant à celui de la deuxième expérience, c'est-à-dire à 95 semaines. Ces rats sont morts entre 20 et 108 jours après le début des injections.

Nous n'avons trouvé, là aussi, qu'un seul animal ayant un carcinome épidermoïde isolé; les autres rats étaient normaux, à l'exception de l'un d'entre eux, mort à $T_0 + 20$ jours (T_0 : début de l'injection), qui présentait un cancer de la vessie; mais étant donné le faible intervalle de temps entre le début des injections et la date de sa mort, et la taille de la tumeur, ce cancer n'a pu être provoqué par la BF.

Pour la cinquième série, nous avons fait inhaler du radon (6000 WLM en 10 semaines) à 40 rats. Nous n'avons fait suivre cette inhalation d'aucun autre traitement.

Ces rats ont eu une survie moyenne plus élevée que celle des séries précédentes, de l'ordre de 570 jours après le début de l'inhalation.

Seize animaux présentaient des cancers pulmonaires de type épidermoïde ou bronchiolo-alvéolaire; les premières tumeurs apparaissent après 430 jours.

Pour les deux dernières séries, nous avons comparé l'action de la benzoflavone à celle d'un autre cancérigène chimique, le méthylcholanthrène, administré à raison

de 12 injections intrapéritonéales (25 mg/kg). Des rats sont morts de 38 à 120 jours après la première injection. Quatre rats présentaient des cancers locaux au point d'injection. Nous avons aussi observé cinq cancers pulmonaires épidermoïdes très comparables à ceux obtenus avec la benzoflavone, très kératinisés mais plus petits.

Les 12 rats de la dernière série n'ont pas inhalé de radon mais ont reçu au même âge que les précédents les mêmes injections de méthylcholanthrène. Ils sont morts de 40 à 140 jours après la première injection, donc dans les mêmes délais que les rats précédents; mais, si là aussi nous obtenons 9 cancers locaux, nous n'avons vu apparaître aucun cancer pulmonaire. Les poumons de ces animaux étaient parfaitement sains, sans la moindre altération anatomo-pathologique.

DISCUSSION

La benzoflavone, administrée seule, n'a permis de retrouver qu'un seul petit carcinome épidermoïde chez deux animaux sacrifiés vers 100 jours. Les poumons des autres animaux, quelle qu'ait été la survie de ces derniers, sont restés parfaitement sains.

Par contre, dès qu'on administre de la BF à des animaux ayant inhalé du radon depuis plus de 5 mois, son effet co-facteur est considérable. Tous les animaux présentent des carcinomes épidermoïdes multiples et très kératinisés.

Ce qu'il faut noter c'est la rapidité du développement des tumeurs et leur spécificité histologique. De plus, il n'y a pas de cancers au point d'injection.

Avec le méthylcholanthrène, on a observé les mêmes tumeurs épidermoïdes apparaissant dans les mêmes délais, mais beaucoup moins développées. De plus, on observe un grand nombre de tumeurs intrapéritonéales.

L'effet co-facteur des deux éléments est donc différent.

La benzoflavone semble se comporter comme un stimulant de la prolifération cellulaire au niveau des bronchioles, l'irradiation intervenant en augmentant le nombre de cellules transformées et en diminuant l'efficacité de la surveillance locale. Pour vérifier cette hypothèse, nous avons lancé de nouvelles expériences en faisant varier les doses des deux facteurs ainsi que les modalités de l'irradiation.

CONCLUSION

Cette expérience montre que l'on peut obtenir, en combinant une irradiation localisée et l'administration d'un produit chimique par voie générale, une augmentation considérable de l'apparition de cancers dans l'organe irradié.

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DISCUSSION

A.B. DORY: Just a point of clarification regarding the dose. Were the animals exposed to a radon concentration of 6000 WL for the period of 12 weeks, or did they receive a total dose of 6000 WLM in 12 weeks?

M. MORIN: It was the total dose received.

J.R. MAISIN: Have you tried administering benzoflavone before exposure to radon?

M. MORIN: No, not yet, but we plan to do this.

ETUDE EXPERIMENTALE DE L'ACTION COMBINEE DE LA FUMEE DE CIGARETTES ET DU DEPOT ACTIF DU RADON-222

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Abstract-Résumé

EXPERIMENTAL STUDY OF THE COMBINED EFFECT OF CIGARETTE SMOKE AND AN ACTIVE BURDEN OF RADON-222.

Previous studies on the carcinogenic effect of radon-222 derivatives have yielded accurate relationships, for each radon dose, between the dose value and the frequencies and latency times of lung cancers. In the present work, one hundred rats were subjected, over one-and-a-half months, to a total dose of 3600 WLM, chosen because it corresponds to a 30% occurrence of cancers. Fifty of these animals then inhaled cigarette smoke during 50 ten-minute sessions per week. The total time for these inhalations was 350 hours spread over about six months. In the 'radon' group (50 rats), 17 animals were found to have lung cancer. In the 'radon + tobacco' group (50 rats), 32 cancers were observed; moreover, the tumours in this group were much more extensive, multifocal and invasive. Animals subjected to cigarette smoke alone have never shown lung cancer. The effect of tobacco as a co-factor in carcinogenesis has thus been verified experimentally, although inhaled cigarette smoke alone is not carcinogenic in rats.

ETUDE EXPERIMENTALE DE L'ACTION COMBINEE DE LA FUMEE DE CIGARETTES ET DU DEPOT ACTIF DU RADON-222.

Les études précédentes sur l'action cancérogène des dérivés du radon-222 ont permis de connaître avec précision les relations qui existent, pour chaque dose de radon, entre la dose, d'une part, et les fréquences et les temps de latence des cancers pulmonaires, d'autre

part. Cent rats ont été soumis un mois et demi à une dose totale de 3600 WLM. Cette valeur a été choisie car elle correspond à l'apparition de 30% de cancers. Cinquante de ces animaux ont ensuite inhalé de la fumée de cigarettes à raison de 50 séances de 10 minutes par semaine; le temps total de ces inhalations a été de 350 heures réparties sur environ six mois. Dans le groupe «radon» (50 rats), on a observé 17 animaux porteurs de cancers pulmonaires. Dans le groupe «radon + tabac» (50 rats), on a observé 32 cancers; de plus, les tumeurs dans ce groupe étaient beaucoup plus volumineuses, multifocales et invasives. Les animaux soumis à la fumée de cigarettes seule n'ont jamais montré de cancers du poumon. L'effet co-facteur du tabac est donc vérifié expérimentalement bien que, chez le rat, la fumée de cigarettes inhalée seule ne soit pas cancérigène.

En exposant des rats à des inhalations de radon-222 et de ses produits de filiation nous avons, au cours d'expériences précédentes, provoqué chez ces animaux l'apparition de cancers du poumon. Ils apportaient la preuve expérimentale de l'effet cancérigène de ce gaz [1, 2].

L'essentiel de nos travaux fut par la suite consacré à l'étude de la relation dose-effet. Les nombreuses et diverses expériences réalisées dans ce but nous ont permis d'observer et de contrôler l'évolution d'environ 500 tumeurs pulmonaires malignes. Elles nous ont également appris à bien maîtriser cette technique de cancérisation. Celle-ci permet notamment de prévoir avec certitude la fréquence et le temps de latence moyen des cancers qui apparaîtront dans une population de rats pour une exposition donnée [3].

Les résultats obtenus ont montré, d'autre part, que ce modèle échappe à certaines des critiques habituellement formulées à l'encontre de l'expérimentation animale. Les cancers du poumon induits de cette façon apparaissent en effet chez des animaux qui n'en font pratiquement jamais spontanément, ils sont histologiquement comparables aux cancers humains, et leur fréquence d'apparition en fonction de l'exposition se superpose à celle qui ressort des enquêtes épidémiologiques sur les cancers du poumon des mineurs d'uranium [4]. Ce dernier point mérite d'être souligné, car il est exceptionnel de pouvoir, à partir de données précises concernant la relation dose-effet, comparer une expérimentation animale à une enquête épidémiologique humaine.

La simplicité et la sûreté de la méthode, la validité du modèle nous ont conduit à envisager différentes sortes d'études et en particulier celle de l'effet co-carcinogène des polluants inhalés. Nous savons en effet que, pour induire un cancer du poumon chez le rat, il faut lui faire subir avec le radon et ses produits de filiation en inhalation une agression cumulée, par gramme de poumon, égale à celle qui est susceptible de provoquer un cancer chez l'homme, et cela pendant la même fraction de vie [5]. Une telle agression est bien tolérée car le radon est un gaz rare dépourvu de toxicité chimique. Cette toxicité interdit par contre un protocole expérimental analogue pour d'autres polluants.

Il faudrait, en effet, les administrer dans des conditions entraînant une intoxication aigüe et la mort de l'animal interviendrait bien avant que le cancer n'apparaisse. Il semble donc a priori très difficile, sinon impossible, de démontrer expérimentalement l'effet cancérigène d'un polluant chimique. En le faisant, par contre, inhaler à concentration sub-toxique à des animaux exposés aussi à une dose déterminée de radon avec son dépôt actif, on pourra au moins apprécier son action co-carcinogène. Celle-ci, d'ailleurs, est peut-être essentielle. Il suffira pour cela de comparer quantitativement et qualitativement les cancers ainsi obtenus à ceux qui sont attendus chez des rats inhalant uniquement la même dose de radon avec son dépôt actif.

Nous avons choisi la fumée de cigarettes dans cette première expérience parce que, si sa relation avec le cancer est indiscutablement démontrée par l'épidémiologie, elle n'a pas encore été clairement confirmée par l'expérimentation animale [6]; également parce que, dans le cadre de la prévention [7] auprès des mineurs d'uranium, il est important de préciser le rôle de ce co-facteur.

MATERIEL ET METHODE

Les inhalations de radon et de ses descendants ont eu lieu dans une installation permettant d'exposer un grand nombre d'animaux. Les chambres d'inhalation et le mécanisme de l'appareil ont précédemment été décrits [8].

Deux lots de 50 rats Sprague Dawley SPF, répartis en cage de dix, ont inhalé dès l'âge de trois mois du radon à l'équilibre avec ses descendants à la concentration de 3000 WL durant 34 séances de 6 heures à raison de 4 séances nocturnes par semaine. L'exposition cumulée a été de 3900 WLM, le pourcentage de cancers du poumon pour une telle dose était environ de 30%.

Pour l'inhalation de la fumée de cigarette, un système simple a été choisi, la boîte à fumée. D'un volume de 500 litres, elle a permis d'exposer ensemble les 50 rats constituant un des lots inhalant du radon. La production de fumée de cigarettes y est assurée par la combustion simultanée de 9 «gauloises bleues». Les cigarettes sont disposées sur un fume-cigarettes communiquant avec la boîte. La combustion est entretenue dès que les cigarettes sont allumées, et l'aspiration de la fumée assurée par une légère dépression créée dans la boîte au moyen d'une pompe à vide. A la fin de chaque séance, une ventilation renouvelle l'atmosphère de l'enceinte en air frais.

La concentration de fumée (9 cigarettes pour 500 litres) a été choisie de telle sorte que les animaux puissent, sans que leur comportement et leur état général s'en ressentent, être soumis à 10 séances d'inhalation de 15 minutes dans une journée, c'est-à-dire au maximum de fumée pendant le maximum de temps. Nous avions pour atteindre ce but procédé par tâtonnement en exposant préalablement quelques rats à des concentrations diverses. C'est ainsi que cinq

d'entre eux avaient bien toléré un total de 500 heures d'inhalation, dans les mêmes conditions. Il est important de noter à ce sujet qu'il n'avait été constaté, à la suite de cette exposition, au niveau de leurs poumons, que des modifications des structures bronchiolo-alvéolaires à type de métaplasie sans aucune lésion maligne [9].

Les rats ont, ici, été soumis pendant un an à 6 à 10 séances d'inhalation de 10 à 15 minutes par jour, 4 fois par semaine durant 176 journées, ce qui fait un total de 352 heures. Pendant les deux premiers mois les rats inhalent la fumée le jour et le radon la nuit.

Cinq rats, deux fumeurs et trois non fumeurs, ne purent être utilisés. Tous les autres, qu'ils soient morts naturellement ou sacrifiés à cause d'un amaigrissement rapide et d'un mauvais état général, furent autopsiés et examinés dans de bonnes conditions et selon le même protocole:

— Tous les organes sont systématiquement examinés et conservés lorsqu'ils présentent la moindre anomalie. Les poumons, prélevés après perfusion en place du système circulatoire pulmonaire avec du sérum physiologique afin d'en chasser le sang, sont dans un premier temps examinés très soigneusement. L'examen macroscopique s'est avéré très important car il donne une idée de la taille de la tumeur et de sa localisation; de plus, une palpation fine du poumon, associée à une vitro-pression sur négatoscope, permet de déceler les tumeurs les plus petites; l'examen histologique, pratiqué par la suite, le confirme.

— Après fixation, les poumons sont coupés de haut en bas transversalement dans un plan frontal en deux parties égales, chacune de ces deux parties est incluse à la paraffine et ensuite systématiquement coupée dans le même sens en coupes de 20 ou de 5 μm . Cette méthode permet le dépistage de la moindre anomalie, même avant coloration. Les coupes de 5 μm retenues sont étalées et colorées à l'hémalum, phloxine, vert alcian, safran.

Afin de pouvoir faire des comparaisons simples et précises, les lésions observées ont été rangées selon une classification dérivée de la classification TNM [10].

T₀: Absence de tumeur

T₁: Présence d'une tumeur de diamètre inférieur à 2 mm

T₂: Présence d'une tumeur de diamètre compris entre 2 et 5 mm

T₃: Présence d'une tumeur de diamètre compris entre 5 et 10 mm

T₄: Présence d'une tumeur de diamètre supérieur à 10 mm

P₀: Pas d'extension à la plèvre

P₁: Extension à la plèvre

N₀: Pas d'atteinte ganglionnaire

N₁: Extension ganglionnaire

M₀: Pas de métastase

TABLEAU I. TABLEAU COMPARATIF

	Cancers	T ₁	T ₂	T ₃	T ₄	P ₁	N ₁	M ₁	M ₂	M ₃
Radon	17	2	4	5	6	10	1	0	4	0
Radon + Fumée	34	2	4	6	22	20	7	0	16	1

M₁: Métastase en dehors de la cavité thoracique

M₂: Métastases intrapulmonaires ou présence de plusieurs tumeurs au niveau des poumons

M₃: Association de M₁ et M₂.

On a ainsi obtenu pour chaque pièce histologique une formule (p. ex. T₄ P₁ N₁ M₀) permettant un classement et des comparaisons statistiques simples entre les rats exposés uniquement au radon et les rats exposés au radon et à la fumée de cigarette (tableau I).

RESULTATS

Nous savions que les inhalations de radon et de ses produits de filiation étaient toujours très bien tolérées; l'inhalation de la fumée de cigarettes surajoutée n'a pas modifié, dans nos conditions expérimentales, le comportement des animaux dont l'état général est resté excellent pendant la durée de l'expérimentation. La durée de vie moyenne des fumeurs a même été plus longue que celle des non fumeurs.

L'examen des poumons a révélé par contre des différences très significatives entre les deux groupes. Tous les poumons des fumeurs ont montré de très nombreuses lésions d'importance variable, souvent au contact des dépôts de goudron, allant de la lésion cellulaire isolée à l'adénomatose. Ces lésions bénignes étaient beaucoup plus rares chez les non fumeurs.

Alors que 17 tumeurs pulmonaires malignes apparaissaient chez les animaux analysés ayant uniquement inhalé du radon et ses descendants, soit environ le pourcentage que nous attendions pour un tel niveau d'exposition — 36% — 34 rats fumeurs, soit 71% des animaux analysés, étaient atteints de cancers du poumon, plus importants et plus invasifs. On compte ainsi 62% de T₄ chez les fumeurs et 41% chez les autres, respectivement 21% et 6% de métastases ganglionnaires (N₁); enfin une métastase à distance chez les fumeurs (M₃),

aucune chez les non fumeurs. De plus la multiplicité des lésions malignes paraissant du même âge dans un même poumon est plus fréquente chez les fumeurs (35% de M₂) que chez les rats inhalant du radon (9% de M₂).

La fumée de tabac ne paraît pas avoir d'influence sensible sur le temps de latence ni sur le type histologique, qui a été comparable dans les deux groupes — environ 75% d'épidermoïdes, 20% d'adénocarcinomes, quelques bronchiolo-alvéolaires et indifférenciés.

DISCUSSION

Malgré l'intensité de l'agression, l'expérience a été menée à son terme dans de bonnes conditions. L'état général des animaux est resté bon et comparable dans les deux groupes. L'inhalation de fumée n'a pas favorisé les infections pulmonaires ni augmenté la mortalité, ce qui tendrait à prouver que les inhalations de radon ne fragilisent pas les animaux. C'est un point très important car cette évolution favorable est indispensable à l'interprétation correcte des résultats.

L'effet co-carcinogène, sur le plan quantitatif, de la fumée de cigarettes a été très clairement démontré, puisqu'il double le nombre de rats cancéreux et multiplie les lésions malignes dans les poumons; de plus, sur le plan qualitatif, les cancers obtenus sont plus invasifs.

Dans cette expérience, où seul le poumon a été significativement irradié, nous n'avons pas constaté chez les fumeurs de fréquence anormale de cancers des autres organes, contrairement à ce qu'on avait observé dans une expérience où la fumée de cigarettes était associée à une inhalation de nitrate d'américium [11].

Il est assez difficile de savoir à combien de cigarettes consommées par un homme correspond l'inhalation de fumée à laquelle les rats ont été soumis. La seule donnée que l'on possède est le taux de l'oxycarbonémie sanguine des animaux lorsqu'ils inhalent de la fumée 0, ml 60%. On pourrait très grossièrement l'estimer à 200 ou 300 paquets au total.

Dans nos conditions expérimentales il est évident qu'il n'y a pas d'addition des risques entre le radon et la fumée de cigarettes, car chez un même nombre d'animaux qui n'ont inhalé que de la fumée de cigarettes nous n'avons jamais observé la moindre lésion maligne.

CONCLUSION

Les constatations précédentes nous paraissent intéressantes à un double point de vue.

Elles mettent en évidence l'action co-carcinogène de la fumée de cigarettes.

Elles vont dans le sens des données épidémiologiques: celles qui concernent les mineurs d'uranium où les facteurs carcinogènes sont les mêmes;

les autres, où, même si le radon n'intervient pas, l'effet du tabac sur l'extériorisation du cancer peut n'être que celui d'un co-facteur, puisqu'il est toujours associé à un ou plusieurs polluants.

Elles apportent une nouvelle preuve de la validité de ce modèle animal qui devrait permettre d'aborder des sujets tels que, par exemple, la nocivité comparée des différents composants de la fumée de tabac, les relations dose-effet, l'efficacité biologique des filtres, mais aussi l'étude des effets co-carcinogènes d'autres polluants chimiques ou physiques.

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DISCUSSION

H. KATO: A possible relationship between radiation and smoking in the induction of lung cancer was examined in the case of the A-bomb survivors and, although the results are not statistically conclusive because of the small numbers involved, it seems that radiation and smoking contribute additively to the induction of lung cancer.

C. PÄUN: Mr. Chameaud, what is the percentage of cancer induction in rats exposed to cigarette smoke alone?

J. CHAMEAUD: We have never observed lung cancer in rats exposed only to tobacco smoke.

A.B. DORY: Are you planning to experiment with the interaction of diesel fumes and radon daughters, considering that diesel fumes can affect the percentage of unattached daughters by increasing the number of condensation nuclei?

J. CHAMEAUD: I am not planning to investigate this personally, but I know that some physicists are interested in the problem.

J.C. EVANS: Have you tried reversing the experiment, so that the animals are exposed first to the cigarette smoke and then to the radon-222? This might then demonstrate the persistence or otherwise of the cocarcinogen smoke, and also perhaps a 'wearing off' of the effects of smoking with time.

J. CHAMEAUD: This is a very interesting question. We have in fact carried out such an experiment with a few animals and they did not get cancer. We cannot draw any conclusions from this, as the number was too small to be statistically significant, but we may repeat the experiment with a larger number of animals.

LOW-LEVEL X-RADIATION-INDUCED ALTERATIONS OF FUNCTIONAL HAEMODYNAMICS IN NORMAL AND DMBA-TREATED, TUMOUR-BEARING HAMSTER CHEEK POUCH EPITHELIUM*

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Abstract

LOW-LEVEL X-RADIATION-INDUCED ALTERATIONS OF FUNCTIONAL HAEMODYNAMICS IN NORMAL AND DMBA-TREATED, TUMOUR-BEARING HAMSTER CHEEK POUCH EPITHELIUM.

Studies previously made by the authors have shown enhancement of 7, 12-dimethylbenz[a]-anthracene (DMBA) tumorigenesis in hamster cheek pouch epithelium by repeated exposures to low-level X-radiation. The aims of the present study were to examine late changes in blood volume and perfusion of cheek pouches treated with either DMBA, repeated low-level X-radiation, or DMBA plus X-radiation, and to relate these vascular changes to the observed tumorigenesis. Hamsters received once weekly 20 R head and neck exposures for 17 consecutive weeks. During weeks 1–5 and 7–11 of irradiation, DMBA was applied to the cheek pouch twice weekly. Appropriate controls were performed. Thirty-nine weeks after treatment initiation, animals received intravenous injections of ^{51}Cr -labelled erythrocytes to determine blood volume and $^{86}\text{RbCl}$ to determine blood perfusion. Pouches and tumours were exteriorized, separately clamped and excised, after which ^{51}Cr and ^{86}Rb activities were determined and histopathological studies were performed. Control and irradiated animals had no tumours, 53% of the DMBA animals had tumours, and 75% of the DMBA plus radiation animals had tumours. Radiation alone caused late increases in blood volume; DMBA or DMBA-induced tumours alone caused late increases in blood volume and perfusion; and DMBA plus radiation caused increases in blood volume similar to those in the radiation only group, and increases in blood perfusion similar to those in the DMBA only group. Whereas DMBA and/or DMBA-induced tumours appeared to control changes in vascular perfusion both with and without the presence of radiation-induced tissue changes, low-level X-radiation appeared to alter or override the tumour or chemically induced changes in blood volume.

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INTRODUCTION

Our laboratory has been studying the effects of repeated low-level x-radiation on chemical carcinogenesis in Syrian hamster cheek pouch epithelium. Thus far, we have studied tumor incidence, latency, histopathology and volume, and tumor and cheek pouch functional vascularity. The 7,12-dimethylbenz[a]anthracene (DMBA)-induced squamous cell carcinoma of the Syrian hamster cheek pouch is an excellent model for such studies since it is a pure epithelial tumor arising from a simple mucous membrane devoid of rete pegs and adnexal structures, its biological progression [1-3] and cell proliferation kinetics [4] are well documented, tumor incidence and latency can be controlled by varying the concentration and application frequency of the carcinogen [5,6], and the cheek pouch itself is highly vascular and can be easily exteriorized and isolated for external irradiation and vascular tracer studies [7,8].

Our previous studies have shown that repeated exposures to low-dose x-radiation significantly enhance DMBA tumorigenesis in Syrian hamster cheek pouch epithelium [9]. Hamsters received either topical applications of 0.1% DMBA in mineral oil, 0.05 ml in the cheek pouch twice weekly for 10 weeks, 20R head and neck x-irradiation once weekly for 17 consecutive weeks, or concurrent DMBA and radiation treatments beginning together. Animals were sacrificed for tumor histopathologic and volumetric studies 45 weeks after the initiation of treatments. The results of these studies are summarized in Table I. The percentage of tumor-bearing animals and the mean tumor volume were significantly greater in animals receiving DMBA plus radiation than in animals receiving DMBA alone. The excess tumors in the DMBA plus radiation group were squamous cell carcinomas. It was suggested that one possible mechanism of the observed radiation enhancement was a radiation-induced alteration in cheek pouch vasculature which resulted in conditions more favorable for tumor induction and growth.

In order to confirm the results of this study and to test the hypothesis that there were radiation-induced alterations in the cheek pouch vasculature associated with the observed increased tumor incidence and size, a second duplicate study was performed in which animals were sacrificed 39 weeks after initiation of treatments, and in which, in addition to histopathologic studies, functional hemodynamic determinations were made on cheek pouch and tumor tissues at sacrifice using fractional distributions of radioisotopes.

MATERIALS AND METHODS

Young adult male Syrian golden hamsters were treated either with DMBA, x-radiation or DMBA plus x-radiation: untreated and sham-treated controls were also included. Irradiation consisted of one 20R exposure to the head and neck per week for 17 consecutive weeks using the following factors: 100 kVp, 200 mA, 1.4 sec exposure, 11 inch TSD, HVL = 1.2mm AL, 1.2mm added Al filtration. These exposures and factors are similar to those used in some diagnostic radiological procedures. Exposures in air were determined using a Victoreen R-chamber, and tissue absorbed doses were determined using lithium fluoride thermoluminescent dosimetry. DMBA treatments consisted of biweekly 0.05 ml topical applications of 0.1% DMBA in mineral oil to the right cheek pouch for 11 consecutive weeks with no applications during week 6. In animals receiving

TABLE I

TUMOR INCIDENCES AND VOLUMES IN HAMSTERS SACRIFICED
45 WEEKS AFTER TREATMENT INITIATION

Treatment	No. Animals	% Papilloma ^a	% Carcinoma ^a	Total No. Tumors	Mean Volume, (mm ³) \pm S.D. ^b
Radiation	9	0	0	0	0
DMBA	20	0	40	43	2.69 \pm 2.79
DMBA + Radiation	19	5	63 ^c	49	7.64 \pm 21.28 ^d

^aPercent of animals in which papilloma or carcinoma was the most severe pathology present. Most animals had multiple small tumors.

^bS.D. = Standard Deviation.

^cLevel of significance vs DMBA: $p < 0.05$ (t-test for statistical differences between two proportions).

^dLevel of significance of mean (\log_e vol.) vs mean (\log_e vol.) DMBA: $p < 0.05$.

DMBA and x-radiation, DMBA treatments were during weeks 1-5 and 7-11 of irradiation, preceding and following radiation exposures by 24 hours. Animals were examined bimonthly for the gross presence of tumors and were sacrificed 39 weeks after the start of treatments for histopathologic and hemodynamic studies.

Fractional distributions of ⁵¹Cr-labeled erythrocytes were used to determine cheek pouch and tumor vascular volumes, and fractional distributions of ⁸⁶RbCl were used to determine cheek pouch and tumor vascular perfusions. Fractional distributions of ⁵¹Cr-labeled erythrocytes and ⁸⁶RbCl have been used to study vascular volume and perfusion in a variety of normal, irradiated and neoplastic tissues [7,8,10-17]. Untreated, irradiated and DMBA-treated tumor-bearing hamster cheek pouches have been studied using these techniques [7,8]. The preparations of these isotopes have been described previously [8]. At the time of sacrifice, with animals under sodium pentobarbital anesthesia, the femoral veins were exposed and the animals received intravenous injections of 20 μ Ci ⁵¹Cr-labeled hamster erythrocytes, followed in 10 minutes by 50 μ Ci ⁸⁶RbCl. Pouches and tumors were everted, clamped separately, and excised separately 90 seconds after ⁸⁶RbCl injection. Tumors were clamped at their bases, first when present, and pouches were clamped adjacent to their exit from the oral cavity. Tissues were weighed and placed into formalin-containing gamma scintillation vials. ⁵¹Cr and ⁸⁶Rb activities were determined by scintillation spectrometry and were then calculated as percent injected dose per mg tissue. Statistical analyses of the data are described subsequently. After radioactivity determinations, tumors were measured, their volumes were determined¹, and all pouch and tumor tissues were prepared for light microscopic histopathological analysis. Serial sections were cut and mounted through the entire dimensions of cheek

¹ Volumes were calculated using the formula: $\text{Vol}(\text{mm}^3) = 0.5326 (A \cdot B \cdot C)$ where A, B and C are the 3 greatest right angle diameters and 0.5326 is the ovoid-spheroid factor [18].

TABLE II

TUMOR INCIDENCES AND VOLUMES IN HAMSTERS SACRIFICED
39 WEEKS AFTER TREATMENT INITIATION

Treatment	No. Animals	% Papilloma ^a	% Carcinoma ^a	Total No. Tumors ^b	Mean Volume (mm ³) ± S.D.
Radiation	14	0	0	0	0
DMBA	15	7	46	8	26.4 ± 37.9
DMBA + Radiation	21	30 ^c	45	12	374.5 ± 881.6 ^d

^aPercent of animals in which papilloma or carcinoma was the most severe pathology present. Most animals had multiple small tumors.

^bTotal tumors are fewer in this study than in the 45 week study because only tumors physically large enough to isolate and clamp were measured.

^cLevel of significance vs. DMBA: $p < 0.05$ (t-test for statistical differences between two proportions).

^dA highly significant difference in variances was found between DMBA and DMBA + Radiation tumors. This was due entirely to the presence of several very large tumors in the DMBA + radiation group which gave rise to the enormous standard deviation.

TABLE III

VASCULAR VOLUME AND VASCULAR PERFUSION IN CHEEK POUCHES AND TUMORS
OF HAMSTERS SACRIFICED 39 WEEKS AFTER TREATMENT INITIATION

There was a significant two-way multivariate interaction between effects of DMBA and x-radiation on ⁵¹Cr and ⁸⁶Rb distributions in the cheek pouches of all treated animals ($p < 0.0007$). A significant one-way multivariate interaction between DMBA and x-radiation ($p < 0.0038$) manifested in the ⁵¹Cr activity ($p < 0.0011$) was found in cheek pouches of hamsters treated with DMBA plus x-radiation.

Treatment	Tissue	N	Radioactivity (% injected dose/mg tissue ± s.d.)	
			⁵¹ Cr	⁸⁶ Rb
Untreated	Pouch	12	0.085 ± 0.046	0.0071 ± 0.002
Radiation	Pouch	14	0.149 ± 0.022 ^b	0.0069 ± 0.002
DMBA	Pouch ^a	8	0.238 ± 0.074 ^{b,c,d}	0.0184 ± 0.008 ^{b,c}
DMBA + Radiation	Pouch ^a	15	0.170 ± 0.044 ^b	0.0197 ± 0.009 ^{b,c}
DMBA	Tumor	8	0.187 ± 0.102	0.048 ± 0.031
DMBA + Radiation	Tumor	12	0.163 ± 0.045	0.080 ± 0.061

^aTumor-bearing pouches only.

^b $p < 0.005$ vs untreated.

^c $p < 0.005$ vs radiation.

^d $p < 0.005$ vs DMBA + radiation.

pouches and tumors. Slides of the tissues were treated as unknowns and were scored for presence and types of tumors by the investigator and an independent pathologist. Pouches usually had multiple areas with pathologic changes: the score a given animal received represented the most severe pathology in that animal.

RESULTS AND DISCUSSION

The results of the histopathologic and volumetric determinations were presented previously [9] and are summarized in Table II. As in the initial study, there were significantly more tumor-bearing animals in the DMBA plus radiation group than in the DMBA group, and the tumors were larger in the DMBA plus radiation group. The excess tumors in the DMBA plus radiation group of this study were papillomas. DMBA-treated cheek pouch tissues follow a predictable, well-documented progression of pathological changes: inflammation, hyperplasia, dysplasia, papilloma, in situ carcinoma and invasive carcinoma [1-3]. Carcinomas invariably arise from the already existing premalignant papillomas [2], and one would expect that some, if not most, of the excess papillomas in the DMBA plus radiation group sacrificed at 39 weeks would have become carcinomas had the animals been allowed to live an additional 6 weeks as in the previous study [9].

The results of the ^{51}Cr and ^{86}Rb distribution study are shown in Table III. Data analysis began with a two-way multivariate analysis of variance [19] to define the possible interaction of DMBA and/or DMBA-induced tumors with x-radiation on cheek pouch vasculature. Following the demonstration of a significant two-way multivariate interaction between radiation and DMBA/tumor effects ($p < 0.0007$), separate one-way analyses of variance were performed to evaluate the effects of radiation vs no radiation on untreated pouches and on DMBA-treated tumor-bearing pouches. Data analysis was then completed with Scheffé testing [20] and t-testing where appropriate.

There were significant increases of blood volume in all experimental groups: the degree of this increase was dependent on the presence or absence of x-radiation. Significant increases of blood perfusion occurred in all tumor-bearing cheek pouches. These increases in perfusion were dependent on earlier DMBA treatments and were independent of the presence or absence of x-radiation treatments. There were no significant differences either in blood volume or perfusion between tumors in the DMBA and DMBA plus radiation groups.

Several investigators have demonstrated substantial functional vascular changes in various tissues following acute exposures of moderate to large doses of x-radiation [7,12,13,16,17,21-25]. These changes have included early increases in vascular volume and perfusion, presumably due to inflammation, increased vascular permeability, sinusoidal pooling and vascular dilation with resultant decreased vascular bed resistance and increased blood flow. In irradiated lung, skin, liver and kidney, these acute changes have been followed by a later return of vascular volume and perfusion to normal levels after moderate doses or reduced levels after large doses [12,24]. However, late returns to normal blood flow or persistent increases of blood flow have been found in irradiated limbs, muscle, skin, brain and hamster cheek pouch epithelium after moderate to large radiation exposures [7,12,13,17,24]. Progressive tissue fibrosis and exuberant obstructive endothelial proliferation in an attempt to repair radiation-damaged vessels have been proposed as mechanisms of late

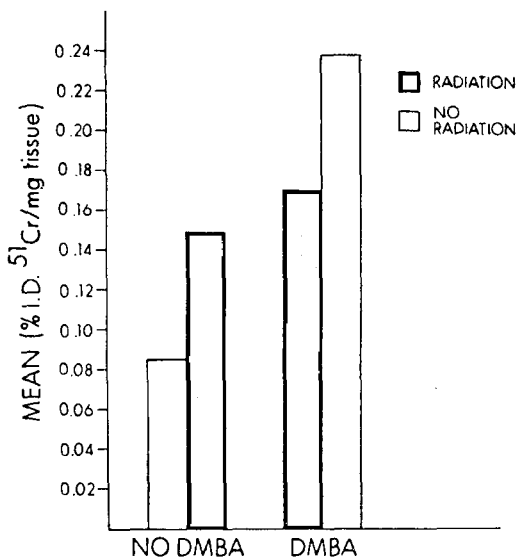


FIG.1. Vascular volumes (^{51}Cr activity) in hamster cheek pouches. DMBA and DMBA plus radiation values are for tumor-bearing pouches only. %I.D. = per cent injected dose. Radiation alone resulted in a significant increase of vascular volume versus untreated controls. Radiation plus DMBA and/or tumors resulted in a vascular volume increase similar to that from radiation alone; however, these values were significantly lower than those seen in tumor-bearing pouches treated solely with DMBA.

reductions in blood volume and perfusion [12,24-27], while latent vasodilatation, increased vascular shunting and increased vascular reactivity to stimuli have been proposed as mechanisms of late increases in blood flow [7,12,17,23]. The late increase of cheek pouch blood volume found in the present study is reconcilable with earlier studies, since the repeated low-level exposures might cause endothelial proliferation with subsequent new vessel growth, as well as persistent vasodilation, without the repair fibrosis and diminished vascular function which follows the severe tissue damage caused by large radiation exposures. A significant correlation was found between changes in ^{51}Cr and ^{86}Rb activities in untreated and radiation only animals ($r = 0.78$). Tissue vascular perfusion was significantly decreased in irradiated animals only when ^{86}Rb values were adjusted for ^{51}Cr values in the multivariate analysis ($p < 0.019$): this enabled irradiated and untreated control pouches of equal ^{51}Cr activity to be identified on the basis of differences in their ^{86}Rb activities. The reduced ^{86}Rb activity is suggestive either of slightly reduced blood flow or of a slight increase in perivascular fibrosis with a resultant diminished efflux of nutrients from the intravascular compartment. The marginal significance of our ^{86}Rb extraction data does not help in clarifying the conflicting data on late post-irradiation changes in vascular perfusion.

In DMBA-treated, tumor-bearing cheek pouches, there were significant increases of blood volume and perfusion. In previous studies employing more vigorous treatments of cheek pouches with DMBA, we had shown that similar but quantitatively larger increases of blood volume and perfusion were associated with the presence of tumors which had volumes greater than 10mm^3 and which were almost invariably carcinomas [8]. We suggested that the increased cheek pouch blood volume and perfusion might have correlated with proliferation of cheek pouch vasculature induced by secretion of tumor angiogenesis factor (TAF) [28,29], and that a direct DMBA effect on the vasculature also might have contributed to these changes. In the present study, ^{51}Cr and ^{86}Rb activities were greater in tumor-bearing pouches than in treated, non-tumor bearing pouches; however, these differences were not statistically significant. This suggests that there is a residual hemodynamic effect from earlier DMBA treatments. Action of chemical carcinogens on epithelial vasculature was further supported by a recent preliminary study in which we found elevated cheek pouch blood volume and perfusion during a treatment course with 0.5% DMBA prior to the appearance of grossly or histologically detectable tumors (Lurie -- unpublished data), and by a study which demonstrated endothelial cell proliferation in rat bladder epithelium during treatment with N-butyl-N-(4-hydroxybutyl)nitrosoamine [30]. Nevertheless, vascular endothelial cell proliferation has been observed in cheek pouch tissue adjacent to DMBA-induced tumors after completion of DMBA applications [31], and cheek pouch vascular proliferation induced by tumor secretion of TAF remains an attractive hypothesis for explaining the slightly higher blood volume and perfusion in the tumor-bearing versus non-tumor-bearing cheek pouches of the present study. Thus, it appears that both DMBA and tumors act upon cheek pouch vasculature. The possibility that DMBA action on vascular endothelial cells affects their subsequent response to TAF stimulation should not be overlooked.

In animals receiving DMBA plus x-radiation, there was a significant multivariate interaction between the effects of x-radiation and DMBA (or DMBA-induced tumors) on the vasculature of tumor-bearing cheek pouches ($p < 0.0038$). This interaction was manifested entirely as an increase in blood volume similar to that found in irradiated pouches but less than that found in DMBA-treated tumor-bearing pouches (Fig. 1). Conversely, increases in tissue perfusion among tumor-bearing pouches of the DMBA plus x-radiation group were similar to that found in DMBA-treated tumor-bearing pouches and greater than that found in irradiated pouches. The similarities of cheek pouch blood volume among radiation only and DMBA plus radiation groups, the similarities of cheek pouch blood perfusion among DMBA and DMBA plus radiation groups, and a low correlation coefficient between changes in ^{51}Cr and ^{86}Rb values in DMBA and DMBA plus radiation groups ($r = 0.12$) suggest that DMBA and/or DMBA-induced tumors govern changes in vascular perfusion while x-radiation exposures govern changes in vascular volume.

We are proposing that repeated low-level x-radiation exposures during DMBA applications result in the alteration or inhibition of a proliferative response of vascular endothelium to DMBA or DMBA-induced tumors, presumably mediated ultimately by TAF. This is a speculative proposal; nonetheless, the highly significant multivariate interaction between radiation and DMBA in the DMBA plus x-radiation group, the essentially equal vascular volumes in irradiated pouches both with and without DMBA

treatments and the presence of tumors, and the low correlation coefficient between ^{51}Cr and ^{86}Rb changes in irradiated and non-irradiated DMBA-treated pouches, are data which are supportive of such a mechanism. Radiation could act directly on vascular endothelial cells by affecting their mechanism of proliferative control, altering or overriding their response to proliferative stimuli from DMBA or TAF. Radiation could also act indirectly on the vasculature by causing a physical barrier-increased perivascular fibrosis — which could interfere with the interaction between endothelial cells and DMBA or TAF. Radiation doses were small, there was no histologically detectable vascular injury or fibrosis, and there was little if any radiation-induced change in vascular perfusion; thus, it appears more likely that irradiated vasculature is unable to proliferate at the time of TAF stimulation or that its proliferative response to DMBA and/or TAF is altered by earlier radiation action.

In conclusion, repeated low-level x-irradiation appears to alter tissue environment in a manner which favors the chemical induction and subsequent growth of tumors. The mechanisms are presently unclear, and may involve radiation effects on the control of vascular endothelial proliferation. Further studies of functional, morphologic and cyto-kinetic vascular changes during carcinogenesis, both with and without the presence of x-radiation, are needed to clarify these mechanisms.

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DISCUSSION

J. BERGSMA: Possibly I have misunderstood you. Enlargement of any organ or tissues is accompanied by an increase in circulation. This also applies to tumours (e.g. in the tumour-bearing pouches), except in massive necrosis. It is not unlikely that in the precancerous phase ('cancer in situ') a local increase in circulation will occur. When you are removing the hamster cheek cancer, it is logical that you should have more scintillations on the monitor. Therefore I do not understand the significance and the consequences of your investigation.

A.G. LURIE: The significance of the blood volume changes in DMBA+ radiation-treated, tumour-bearing cheek pouches lies in the fact that the low-dose X-radiation appears to have overridden the effects of tumours on the tumour bed vascular volume. These tumours, when induced by DMBA alone, cause a great increase in vascular volume of the pouch tissue, whereas the blood volume increases in tumour-bearing pouches treated with DMBA plus radiation were equal to those seen in pouches treated with radiation only, which had no tumours, and were significantly less than those seen in pouches treated with DMBA only. Thus, radiation effects on vascular volume appear to have altered or blocked the known angiogenic effects of tumours on tumour bed vasculature.

C. STREFFER (*Chairman*): You suggested that mitogenic effects could contribute to the combined effect. Is there any experimental evidence of mitogenic effects from DMBA?

A.G. LURIE: The only study I am aware of on this subject is that of McKinney, Singh and Kolas (Ref. [31] of the paper), who investigated DMBA-treated, tumour-bearing cheek pouches and demonstrated, autoradiographically, areas of increased labelling indices of vascular endothelial cells in cheek pouch tissue adjacent to tumours. I would add that radiation-induced vascular proliferation is only one possible explanation for our findings. More detailed, morphometric studies are needed to examine the possibility of increased vessel sizes.

DNA REPAIR AND THE ASSESSMENT OF THE BIOLOGICAL HAZARDS OF IONIZING RADIATION

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Presented by G. Cowper

Abstract

DNA REPAIR AND THE ASSESSMENT OF THE BIOLOGICAL HAZARDS OF IONIZING RADIATION.

The somatic (carcinogenic) and genetic hazards of ionizing radiation are both thought to be related to a very small residue of the initial DNA damage which remains after DNA repair processes have run their course. Host factors or environmental factors which interfere with DNA repair processes might therefore be expected to have a marked influence upon the biological effects of a given dose of radiation. Experiments on underlying mechanisms have been carried out to assess the impact of DNA repair processes upon estimates of risk. Hereditary defects in the DNA repair systems occur naturally in human populations; the rare disease ataxia telangiectasia is a notable example. Persons who are heterozygous for this hereditary defect may also be more radiosensitive than normal. Exogenous agents which interfere with the repair of radiation damage to DNA may potentiate radiation effects in one test system but not in another. An assay based on genetic events produced by the recombinational repair of DNA damage in yeast appears to be useful as an indicator of the type of damage responsible for carcinogenic action of ionizing radiation. The data taken together suggest that it is reasonable to assess the hazards of low-level radiation by linear extrapolation from measured effects at high radiation levels.

1. INTRODUCTION

The biological hazards of low-level radiation are generally considered to be restricted to the induction of cancers and of genetic defects [1-3]. These hazards are thought to be largely a consequence of radiation damage to DNA [4-7]. Different types of radiation damage to DNA can be measured by

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a variety of physico-chemical techniques; however, most of the initial damage disappears soon after irradiation as a result of enzymatic repair systems in the living organism [7, 8]. It seems reasonable to conclude, therefore, that the late effects of radiation are ultimately due to a very small portion of the initial damage which is either repaired incorrectly or not repaired. Any factor which affects the DNA repair systems might thus be expected to alter the magnitude of the biological hazards of radiation.

The present report describes some recent experiments in our laboratory on the effects of DNA repair systems in various organisms and discusses the relevance of the findings to assessment of radiation hazards.

2. HEREDITARY DEFECTS IN DNA REPAIR

Hereditary deficiencies in the enzymatic DNA repair systems have been studied in micro-organisms for many years [8], and much of our current knowledge of DNA repair mechanisms stems from these studies. The first established example of a hereditary defect in DNA repair in human populations was xeroderma pigmentosum, a rare disease primarily characterized by hypersensitivity to the ultraviolet (u.v.) component of sunlight [7]. Recently the rare hereditary disease ataxia telangiectasia (AT) has been shown to involve defective repair of DNA damage induced by gamma-radiation [9–11]. This disease

TABLE I. RADIATION SENSITIVITY OF CULTURED HUMAN FIBROBLASTS FROM NORMAL PERSONS, AT PATIENTS AND AT HETEROZYGOTES

Radiation	Gas phase	Radiation dose ^a that reduces survival to 10% (rads)		
		Control	AT heterozygotes	AT
Gamma rays	N ₂	630 (550–720)	465 (335–640)	185 (160–220)
Gamma rays	O ₂	375	—	115
14 MeV neutrons	O ₂	120	—	85

^a Values in brackets indicate the range of 10% survival dose in cases where five or more independent strains in a particular category have been tested. The non-bracketed AT values are for strain AT3BI; the non-bracketed control values are for strain CRL 1141; and the non-bracketed value for AT heterozygotes is the average for five independent strains.

TABLE II. INITIAL YIELD OF DIFFERENT TYPES OF DAMAGE TO DNA IN CULTURED HUMAN DIPLOID SKIN CELLS (FIBROBLASTS) EXPOSED TO GAMMA RADIATION

Type of damage	Approximate yield/100 eV	
	O ₂	N ₂
'Overt' single-strand breaks	2.4	0.9
Alkali-labile bonds ^a	0.6	0.5
Base damage detected with <i>Micrococcus luteus</i> endonucleases	1.6	2.0
Damage detected with purified endonuclease S ₁ from <i>Aspergillus oryzae</i>	1.0	0.7
Double-strand breaks ^b	0.15	0.04

^a Single-strand breaks are usually measured together with alkali-labile bonds; the percentage contribution from alkali-labile bonds is based on previous work with *E. coli* [14].

^b Data on double-strand breaks are based on our own experiments with *M. radiodurans* [15]. The value from other experiments with fibroblasts [16] is about half the above value.

TABLE III. DISAPPEARANCE OF RADIATION DAMAGE FROM THE DNA OF CULTURED HUMAN FIBROBLASTS AFTER EXPOSURE TO 50 krad ⁶⁰Co GAMMA RADIATION UNDER NITROGEN

Type of damage	Time of repair (hours)	% damage remaining ^a	
		Control cells	AT3BI cells
Single-strand breaks plus alkali-labile bonds	1.5	20	22
Base defects detected by <i>M. luteus</i> endonucleases	3.0	21	70
Damage detected by endonuclease S ₁	0.5	5	85

^a Data other than those with endonuclease S₁ are adapted from reference [9]. Pyrimidine dimers disappear from the DNA of strain AT3BI at a normal rate [9]. Repair of double-strand breaks appears to be normal in all AT strains tested to date [16].

is associated with a high risk of cancer, notably lymphoreticular neoplasms. AT patients are known to be radiosensitive; this radiosensitivity can also be demonstrated with cultured AT skin cells *in vitro* [11, 12]. At the level of 10% survival, the cells from AT patients are three to four times more sensitive than normal to acute exposures to gamma radiation, but only 1.4 times more sensitive to neutrons (Table I). These data are consistent with the fact that a large fraction of the DNA damage caused by neutrons is not repaired [13].

The yield of several classes of gamma-radiation damage to cellular DNA is summarized in Table II. Most of the initial radiation damage to the DNA of these cells appears to be repaired fairly rapidly (Table III), even after exposure to high doses of gamma radiation that are ultimately lethal to the cells (Table I). The AT syndrome is associated in most cases with deficiencies in the excision repair of base defects and, more specifically, with the repair of damage detected by endonuclease S_1 , an enzyme which attacks preferentially single-stranded DNA or regions of large distortions in the normal duplex structure of DNA (Table III). The exact chemical nature of this damage is still unknown.

AT individuals are homozygous, that is, they carry two defective copies of an autosomal recessive gene; they represent a very small fraction of the human population, roughly one in 40 000 persons at birth [17]. It can therefore be estimated that heterozygotes carrying one copy of a mutated AT gene constitute one in 100 persons [17]. These heterozygotes exhibit a modest increase in risk of cancer induction [17]. Cultured cells from about half the AT heterozygotes tested to date are intermediate in radiosensitivity between those from normal persons and those from AT patients (Table I). The radiosensitivity is correlated with ability to carry out DNA repair replication; those heterozygous strains which are most radiosensitive exhibit the greatest deficiency in repair replication [11]. Four of the five heterozygous strains tested to date show a linear or 'one-hit' dose-effect curve for cell survival after acute gamma radiation under N_2 [18]. A one-hit survival is also characteristic of cells from AT homozygotes [11, 12]. In contrast, all of the control strains exhibit a pronounced shoulder on the dose-survival curve with acute gamma radiation [11, 12] and, in keeping with theory [19], should therefore be considerably less sensitive to high doses of chronic gamma radiation.

Certain types of hereditary retinoblastoma are also associated with increased radiosensitivity *in vitro* [11, 20]. Other genetic diseases in humans are suspected to involve some abnormality in response either to ultraviolet or to ionizing radiation [7, 11]. It is estimated that 30 or more distinct genes affect DNA repair processes in micro-organisms [21]. At least six mutated loci have been implicated in xeroderma pigmentosum [7], and at least three in AT [10]. Thus it is not unreasonable to expect at least 30 genes involved in DNA repair in humans, in which case a substantial portion of the total population, possibly as much as 10% [22], might be heterozygous for some genetic defect in DNA repair. The implications will be discussed in Section 5.

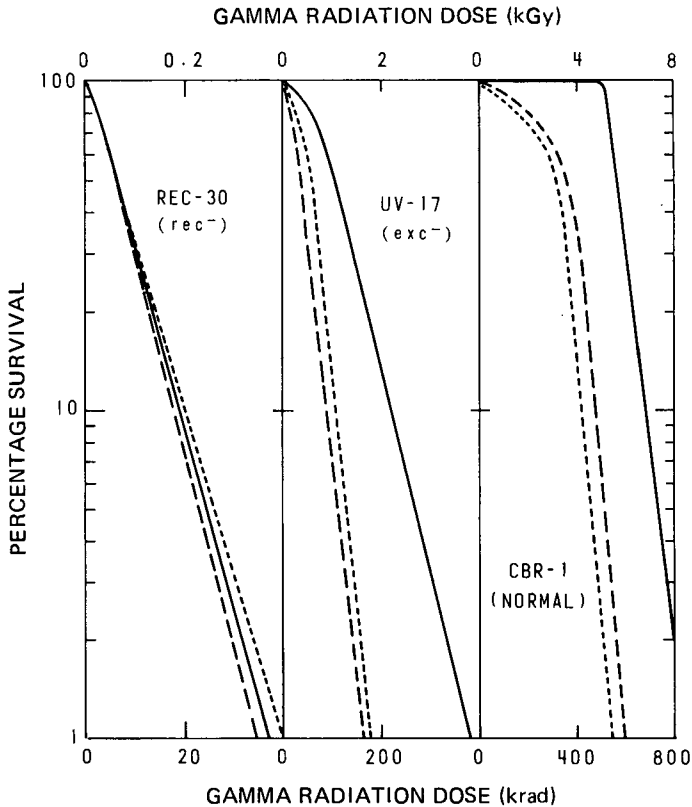


FIG.1. Dose-survival curves for three strains of *M. radiodurans* when plated on normal medium (solid line), medium containing 0.5 mg caffeine/ml (dashed line) and medium containing 0.25 µg acriflavine/ml (dotted line) after exposure to ^{60}Co gamma radiation at a dose rate of 22 krad/min.

3. SYNERGISTIC INTERACTIONS WITH OTHER AGENTS

Inhibition of a DNA repair process with chemicals such as caffeine or acriflavine causes an appreciable increase in the lethal effects of gamma radiation on micro-organisms (Fig.1). Since this enhancement is most marked in mutant strains which depend mainly on recombinational repair, one of the two principal repair pathways, and is absent in mutant strains which depend mainly on excision repair, the other major repair pathway (Fig.1), the effects are thought to be mediated via the recombinational system. Expression of recombinational repair activity depends at least partially on post-irradiation protein synthesis in *Micrococcus radiodurans*; thus chemicals such as chloramphenicol which inhibit

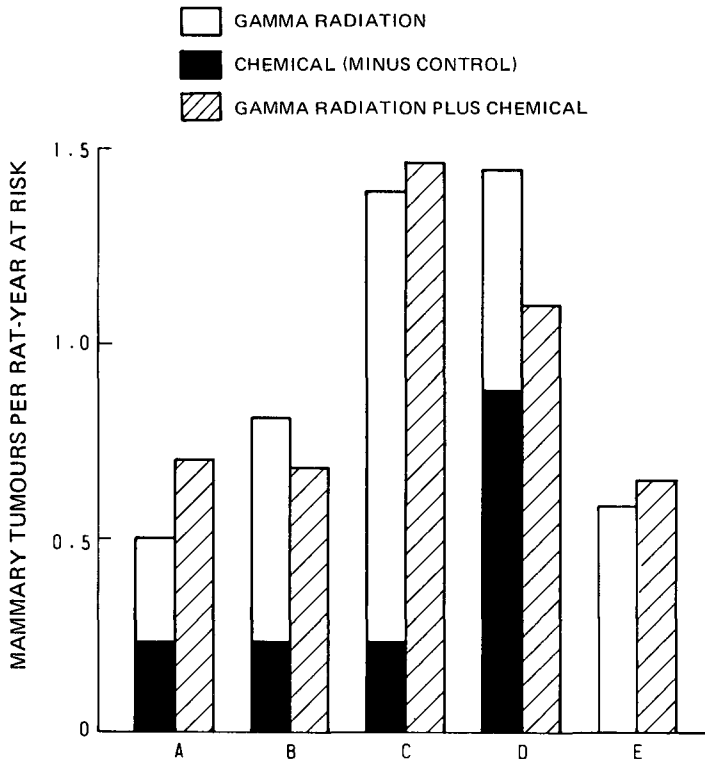


FIG.2. Mammary tumours induced in female Sprague-Dawley rats within one year after exposure to ^{60}Co gamma radiation (dose rate 6 rads/min). In the first three experiments above, animals were injected intraperitoneally with 4 weekly doses of 0.5 g urethane/kg commencing 1 week after irradiation (total dose 2 g/kg); the radiation doses were 50, 100 and 200 rads in experiments A, B and C respectively. Experiment D was carried out similarly with a radiation dose of 100 rads and 4 weekly doses of 1 g urethane/kg (total dose 4 g/kg). In experiment E, animals were injected intraperitoneally with 0.1 g caffeine/kg at the time of irradiation, at 5 hours and at 23 hours after irradiation with 100 rads. Caffeine alone did not induce any excess of mammary tumours over those found in control animals.

protein synthesis also increase the lethal effects of radiation. Enhancement of the effects of gamma radiation on *Schizosaccharomyces pombe* by caffeine similarly appears to depend on inhibition of a recombinational repair system analogous to that responsible for sister chromatid exchanges in mammalian cells [23].

Synergistic interactions between gamma radiation and u.v. radiation have also been demonstrated in *S. pombe* [24]. This synergism does not occur in a radiosensitive mutant defective in recombinational repair, and appears thus to be caused by overloading of the recombinational repair system with different

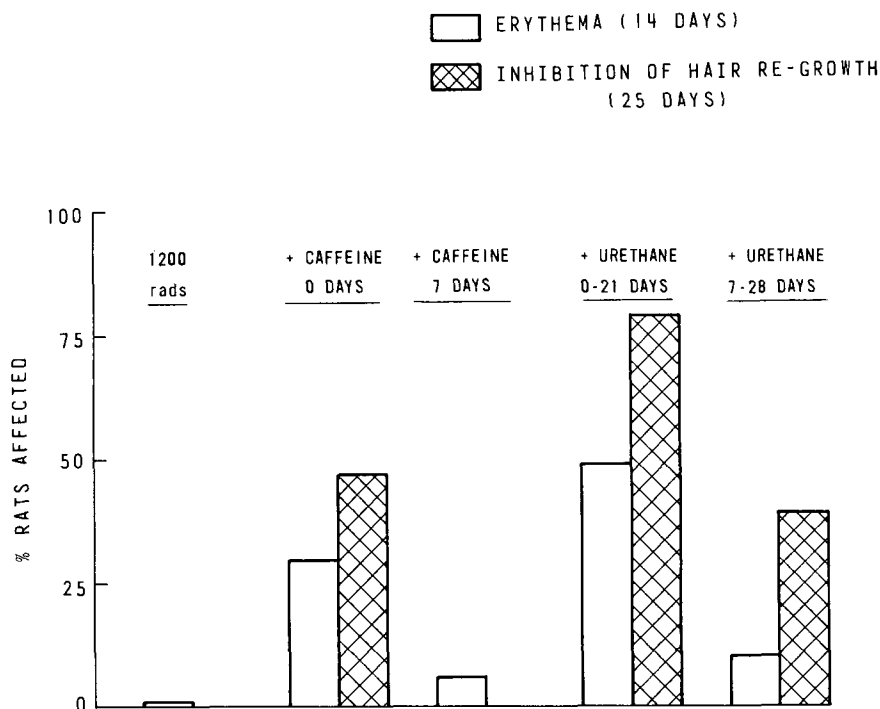


FIG.3. Initial reactions of rat skin to 1200 rads of ^{90}Sr - ^{90}Y beta radiation (dose rate 700 rads/min at skin surface). Hair on the back of male Sprague-Dawley rats, age 2 months, was shaved and a 1 by 3 cm rectangular area exposed to beta radiation. Two groups of animals, each consisting of 36–38 rats, were injected with three doses of 0.1 g caffeine/kg at intervals of 0, 5 and 23 hours, commencing either at the time of irradiation (cf. Fig.2) or 1 week later. Two other groups were injected with four doses of 1 g urethane/kg at intervals of 0, 1, 2 and 3 weeks, commencing either at the time of irradiation or 1 week later (cf. Fig.2). Caffeine alone or urethane alone did not produce erythema.

types of DNA damage arising from exposure to u.v. and gamma radiation. Presumably a similar synergism could occur with other carcinogenic agents.

Studies were undertaken with animals to explore the possibility that similar interactions might enhance the carcinogenic activity of gamma radiation. These radiation studies have concentrated on caffeine, a putative inhibitor of DNA repair, and urethane, a known carcinogen [25] and promoting agent which potentiates the leukaemogenic effect of radiation in mice [26].

The number of mammary tumours appearing in female rats at an early age is directly proportional to the total dose of gamma radiation [27], and is scarcely affected by fractionation of the dose [28], suggesting that the effect is dependent on a 'one-hit' process. In our experiments, the number of mammary

tumours was not increased by injection of caffeine in large amounts immediately after the animals were exposed to gamma radiation; the effects of urethane plus gamma radiation were not greater than additive (Fig.2). In contrast to the results with mammary tumours, the number of skin tumours per rad induced by acute radiation increases markedly with radiation dose [29], and is markedly reduced by dose fractionation [30]. The initial response of the skin to acute beta radiation is markedly potentiated by injection of caffeine or urethane at the time of irradiation (Fig.3). When a week was interposed to permit completion of repair processes after irradiation, subsequent injection of caffeine had no effect and potentiation by urethane was considerably reduced (Fig.3). Data on numbers of skin tumours are not yet available for these experiments, but there is reason to believe that these numbers will be related to initial effects [31]. Moreover, it is well established that cigarette smoke condensate [32] and other agents will increase the number of skin tumours induced by radiation.

Thus, the same chemical agents may appear to potentiate radiation effects in one system (Fig.3) but not in another (Fig.2). In order to obtain some indication of the overall effects on the whole animal, we have also carried out experiments with high doses of X-radiation and of urethane. The results obtained with three different strains of rats indicated that the overall carcinogenic and life-shortening effects of radiation plus urethane are not greater than additive [33]. Results with a fourth, Fischer strain of rat have confirmed this conclusion.

4. GENE CONVERSION ASSAY FOR DETECTION OF CARCINOGENS

A point mutation assay in *Salmonella*, commonly known as the Ames' test [5], has proved to be extremely valuable as a rapid screening procedure for the carcinogenic potential of many chemical agents. A small number of known carcinogens including ionizing radiation do not fit into the general pattern of a correlation between mutagenic activity in *Salmonella* and carcinogenic activity in animals [6, 34].

We are currently exploring a gene conversion assay in D7 strains of yeast [35] as an indicator of genetic damage from ionizing radiation and other carcinogenic agents which are relatively ineffective in the *Salmonella* mutation assay. Gene conversion is essentially a test for recombinational events occurring within a specific gene, and can presumably be induced by a single damaged site within the gene.

Significant increases in gene conversion are demonstrable with acute X- or gamma-radiation doses as low as five rads in strain D7-52a in O₂ (Fig.4). The response appears to be linearly related to dose, and does not begin

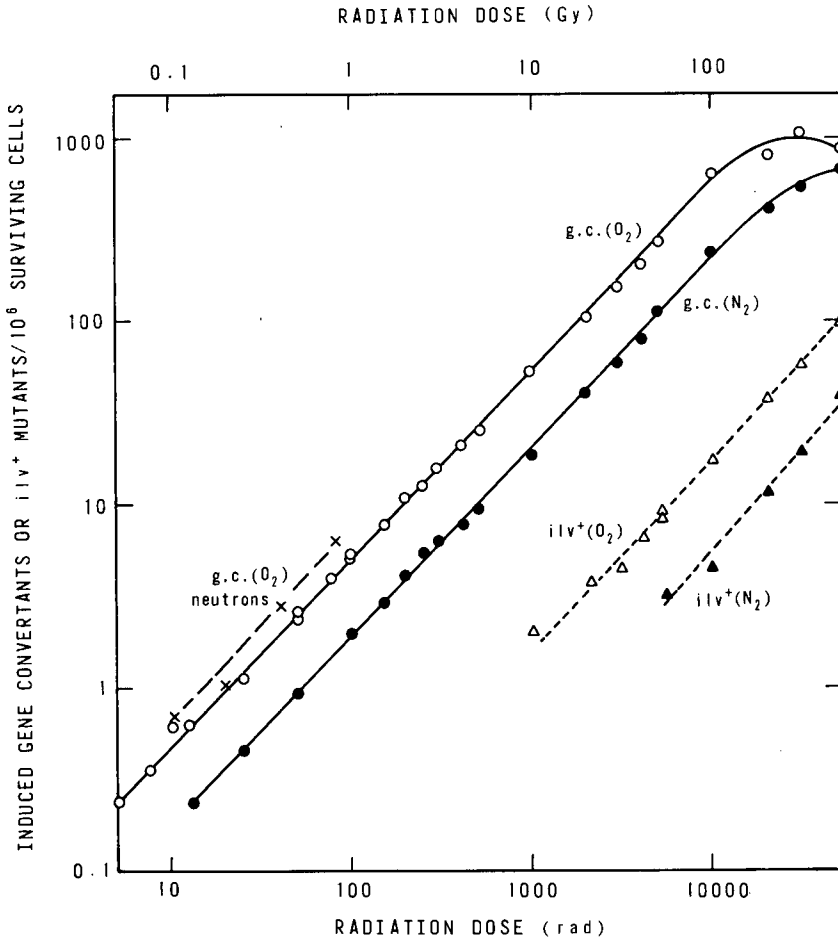


FIG.4. Gene conversions and isoleucine-valine mutations induced in the heterozygous D7-52a strain of *Saccharomyces cerevisiae* after exposure to radiation in the presence of oxygen or nitrogen. Late log-phase cells were irradiated at 0°C with 14 MeV neutrons, with 150 kVp X-rays or with ^{60}Co gamma rays at a variety of dose rates varying from 1 up to 22 000 rads/min. Data for X-ray and gamma irradiation with a wide variety of dose rates at 0°C all appeared to fall on the same line. Neutron irradiation in N_2 was 1.35 times less effective than in O_2 for induction of gene conversion; the data are not shown. The isoleucine allele is suppressible; the ilv^+ revertants may therefore result from mutation at any one of a number of different loci [35]. Thus the average yield of ilv^+ mutations per gene should be about one-tenth of the total values shown above.

to saturate until the dose exceeds 10 000 rads (Fig.4). Neutrons are only 1.3 times more effective than X-radiation in O_2 (Fig.4). The dose-response relationships for the heterozygous D7-52a strain are similar to those for the normal D7 strain; the D7-52a strain was used because the background level of gene conversions is extremely low. An assay for mutations affecting the isoleucine-valine locus was much less sensitive to gamma radiation than the gene conversion assay, even when using the same cell strain for both assays (Fig.4). Further experiments with repair-deficient D7 strains have shown that the point mutation and the gene conversion assays must be mediated by different repair pathways in these strains.

The gene conversion assay is sensitive to u.v. radiation and a number of chemical carcinogens [6] as well as to ionizing radiation (Fig.4). This assay does seem to represent a more reliable and more sensitive indicator of the carcinogenic potential of ionizing radiation than do assays based on the induction of point mutations, and may thus provide information on the type of DNA damage associated with cancer induction.

5. ASSESSMENT OF RADIATION HAZARDS

The carcinogenic effects of normal environmental levels of radiation cannot be measured directly in human populations [36], and are therefore conventionally estimated by linear extrapolation from measured effects of high-level radiation [1, 3]. It is generally acknowledged that this extrapolation provides a safety factor in assessment of the risks of low-level gamma radiation [1-3]. Empirical data from animal experiments in which the effects of chronic and acute exposures were compared suggest that the safety factor is about five-fold on the average [37], with a wide range of values depending upon the end-point involved.

Since the animals used for these studies were inbred, these experiments do not make any allowance for the existence of radiosensitive mutants as part of the total population. Homozygous radiosensitive individuals suffering from diseases such as AT are extremely rare in human populations. However, heterozygous carriers of a defective DNA repair gene are much more common. Cells from many of the AT heterozygotes, in contrast to those from normal individuals, exhibit a linear or nearly linear 'one-hit' dose-effect curve for survival after exposure to acute gamma radiation [19]. It might therefore be expected that the safety factor involved in the extrapolation from acute to chronic gamma radiation would be very small or non-existent for these particular individuals [20].

Assuming that 90% of the total population is five times less sensitive to the carcinogenic effects of chronic than to those of acute gamma radiation, the

linear extrapolation model does, however, still provide a net overestimate of the effects of low-level gamma radiation on the total population. As a hypothetical example, if 1 rem of acute gamma radiation induced 1 fatal cancer/ 10^4 normal homozygotes (carrying no mutations in genes for DNA repair) in the human population (cf. Ref. [1]), then the same dose of chronic gamma radiation might induce about 1.4 cancers/ 10^4 AT heterozygotes, 0.2 cancers/ 10^4 normal homozygotes and 0.32 cancers/ 10^4 persons in a mixed population consisting of 90% normal homozygotes and 10% repair-deficient heterozygotes with a radiation sensitivity equal to that of AT heterozygotes. The heterozygotes with deficiencies in some aspect of DNA repair would presumably be hypersensitive to a number of chemical carcinogens as well as to gamma radiation.

The difference in sensitivity of cells from normal individuals and AT patients to neutron radiation is relatively small (Table I). Thus the presence of AT heterozygotes or similar heterozygous DNA repair mutants in human populations should have little effect on assessment of the biological hazards of low-level neutron radiation or other densely ionizing radiations.

Synergistic interactions of radiation with other environmental agents (Figs 1–3) might appear to pose more of a problem for risk assessments, and continued attention to this area seems warranted. However, any synergistic interactions occurring in human populations as they have lived over the past 30–40 years are already taken into account in the accepted estimates of carcinogenic hazards of ionizing radiation to humans [1–3].

Altogether, it seems reasonable to conclude that carcinogenic risk estimates based on a linear dose-effect relationship provide some safety factor for the major portion of the population in the case of sparsely ionizing radiation. However, the discovery of radiosensitive subgroups in the human population and the possibility of synergistic interactions between radiation and new environmental agents argues against any major reduction in current risk estimates for chronic gamma radiation.

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DISCUSSION

K.H. CHADWICK: Could you tell us what time delay occurred between irradiation and urethane treatment in the induction of mammary cancers, and whether this one-hit effect is very sensitive to radiation?

G. COWPER: The animals were held for one year after treatment with urethane and/or radiation in the mammary tumour experiments. A whole-body dose of about 170 rads of gamma radiation was required to induce an average of one mammary tumour per rat-year at risk in these animals.

C. STREFFER (*Chairman*): At the IAEA-NEA sponsored symposium on the combined effects on the environment of radioactive, chemical and thermal releases from the nuclear industry, held in Stockholm in 1975, I also made the suggestion on the basis of experimental data that repair or recovery from radiation damage is impaired in many interaction mechanisms between radiation and various substances, and that in these cases the linear-dose relationship for risk estimates is generally still 'safe', as the observed dose effect narrows under the modifying effect of the substance to a linear relationship. Thus, I am glad that we agree on this aspect. However, this means that the dose-modifying factor is dependent on the dose of the substance as well as the radiation (see STREFFER, C., VAN BEUNINGEN, D., MOLLS, M., PON, A., SCHULZ, S., ZAMBOGLOU, N., "In vitro culture of pre-implanted mouse embryos – a model system for studying combined effects", these Proceedings 2, IAEA-SM-224/801). Now I should like to put a question: Were the experiments which you reported also performed with different doses?

G. COWPER: Yes. Most of these experiments involved different doses. For example, the data on acceleration of the appearance of mammary tumours involved three radiation doses at one particular dose of urethane and two doses of urethane at one radiation dose (plus appropriate controls).

GENERAL DISCUSSION

A. ARSENAULT: In the light of the questions raised in the paper presented by Cowper (SM-224/807), and in view also of the problem of the statistical independence of observations that we have been discussing, it would be interesting to include as a co-variable in epidemiological studies a parameter indicative of the family prevalence of cancer, in order to test the hypothesis according to which the probability of occurrence of a cancer in an exposed worker would be a function both of the family prevalence and of exposure to radiation. For control purposes, one could then compare results with the prevalence of cancers in families at risk but not exposed to radiation.

H. ALTMANN: I believe that your suggestion could be of considerable value in approaching the question of people with a high risk of cancer working in the nuclear industry. DNA repair capacity is certainly an important factor in the 'resistance' of man to different influences, including radiation. Ataxia telangiectasia is not the only disease that has a DNA repair defect so that DNA damage produced by ionizing radiation cannot be completely repaired. Down's syndrome, systematic lupus erythematosus, Fanconi's anaemia, Bloom's syndrome, scleroderma, rheumatoid arthritis, etc., also show some DNA repair imbalances. Therefore, further work, including family studies, are necessary to solve this interesting and important question.

K.H. CHADWICK: I should like to support this idea of looking for a family history of cancer in epidemiological studies on workers in the atomic energy industry. I'm not sure if this information would be available but if it is, it may provide some pointers.

CHEMICAL PROTECTION

Session 9, Part 2

A COMPARATIVE STUDY OF THE LATE SOMATIC EFFECTS OF WHOLE-BODY X-IRRADIATION AND RADIATION PROTECTION BY AMINOETHYLISOTHIOURONIUM (AET) AND 5-HYDROXYTRYPTAMINE (5-HT) IN INBRED FEMALE SWISS MICE

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Abstract

A COMPARATIVE STUDY OF THE LATE SOMATIC EFFECTS OF WHOLE-BODY X-IRRADIATION AND RADIATION PROTECTION BY AMINOETHYLISOTHIOURONIUM (AET) AND 5-HYDROXYTRYPTAMINE (5-HT) IN INBRED FEMALE SWISS MICE.

The late somatic effects of sublethal whole-body X-ray doses of 400 R, 200 R, 100 R (250 kV, 2 mm Al filter, 100 R/min) and the effects of AET and 5-HT against 400 R were investigated in 42 – 45-days-old inbred female Swiss mice. AET (300 mg/kg body weight) or 5-HT (75 mg/kg body weight) was injected intraperitoneally ten minutes before irradiation. A very high standard of animal maintenance and care in experimental work was observed. Life-span and incidence of neoplasms at death were studied. The late effects observed were reduction in life-span, incidence of thymic lymphoma, myeloid leukaemia, mammary and ovarian tumours. Lung tumours and uterine tumours were also observed. Dose-effect and time-effect relationships between the protected and unprotected groups are presented. The quantitative, and also certain qualitative differences, between the effects of AET and 5-HT are discussed.

1. INTRODUCTION

There are a number of reports on the induction of cancer in rats and mice following exposure to sublethal doses of X- and gamma radiation and also neutrons [1 – 6]. It is also well established that a number of radioprotective chemicals are known to increase markedly the short-term survival of X-irradiated animals. However, the usefulness of these chemicals on the overall life span shortening and on the increased incidence of neoplasms is limited. A few reports [7 – 12] have shown conflicting results. This study discusses the results of our work with aminoethylisothiouromium (AET) and 5-hydroxytryptamine (5-HT) on mice.

2. MATERIALS AND METHODS

Swiss mice, maintained by brother-sister mating for over 26 generations from our animal colony, were used. Spontaneous incidence of diseases and longevity are clearly known as these aspects were repeatedly studied in the breeder stock. A very low incidence of spontaneous neoplasms suggested the suitability of this strain for the study. Moreover, it was felt that the inbred character would ensure uniformity of responses to treatments.

2.1. Animal maintenance and care

The mice were maintained on a standardized laboratory diet and water was provided *ad libitum*. The animals were housed in batches of four to a cage. The room temperature was maintained at 22° – 25°C and humidity at 45 – 50%. Cages, accessories and bedding material (rice husk) were always sterilized before use. Measures to guard against sources of infection were taken.

2.2. Irradiation and injection

The animals were 42 – 45 days old at the beginning of the experiment, and they were randomly assigned to experimental and control groups. All the experimental groups had 50 animals per group and the group given 5-HT had 25 animals.

The radiation was given with a deep X-ray machine (Siemens, stabilipan) at 250 kV, 2 mm Al filter, at 100 R/min. The dose was measured with a victoreen dose meter. The animals were confined in batches of ten in a specially designed box. All measurements were made at mid point of the box.

AET and 5-HT were procured from the Sigma Chemical Company. Freshly prepared neutral solutions of AET (0.75%) and 5-HT (0.1875%) were used for injections. AET was injected at a dose of 300 mg and 5-HT at 75 mg per kg body weight. Injections were made intraperitoneally 10 minutes before irradiation.

2.3. Experimental procedure

The cages were examined daily and any mouse showing signs of sickness, weakness or abnormality was isolated from cagemates and was kept under special observation. When it was found that the animal was nearing death, blood examination was conducted with blood obtained from the tail-tip. Total leucocyte counts were taken. Blood smears were stained with Giemsa stain and examined. When moribund, the mice were mostly sacrificed. Each mouse was necropsied and visually detectable abnormalities were recorded. Specimens

needed for histological examination were collected in 10% formalin. Permanent slides stained with haematoxylin and eosin were prepared and examined.

2.4. Short-term survival

Experiments were conducted to determine the LD₅₀ (30 days) for AET-protected, 5-HT-protected and unprotected groups of mice.

The value arrived at was 575 R for mice treated with radiation alone, 989 R for the AET group and 845 R for the 5-HT group. On comparing the LD₅₀ values, the dose-reduction factor is approximately 1.72 for AET and 1.47 for 5-HT protection. The sublethal doses of 400 R, 200 R and 100 R used to study the late effects are nearly 70%, 35% and 17.5% respectively of the LD₅₀ value.

3. LATE SOMATIC EFFECTS

3.1. Effect on life-span (Figs 1 and 2, Table I)

The reduction in life-span was 68% in the 400 R, 44% in the 200 R and 29% in the 100 R group, as against 49% in the AET + 400 R and 54% in the 5-HT + 400 R group.

Reduction in life-span after excluding thymic lymphoma and early myeloid leukaemia deaths was 48% in the 400 R group, 34% in the 200 R and 26% in the 100 R, as against 36% in the AET + 400 R and 42% in the 5-HT + 400 R group.

The mean life-span of animals treated with AET before 400 R exposure corresponds to a dose of 240 R and those receiving 5-HT to a radiation dose of 280 R in unprotected animals. If, however, early deaths due to thymic lymphoma and myeloid leukaemia are excluded, the 5-HT group corresponds to 310 R in unprotected animals. On the basis of the above a dose reduction factor of about 1.66 for AET and approximately 1.43 for 5-HT could be calculated for long-term survival. These values are similar to those obtained for short-term survival.

3.2. Total neoplasm

The percentage of animals carrying one or more neoplasms at the time of death for each experimental group is presented in Table II.

Whereas the total numbers of animals carrying neoplasms in the irradiated and radioprotected and irradiated groups are not significantly different, these values are markedly increased compared with the control group. However, among the irradiated groups there are distinct differences when one takes into account the life-span of these animals and also in the spectrum of neoplasms.

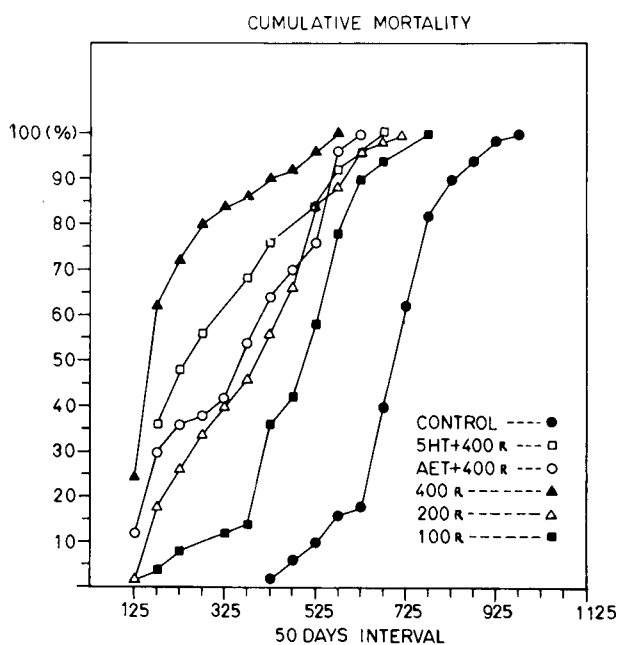


FIG.1. Effect on life-span.

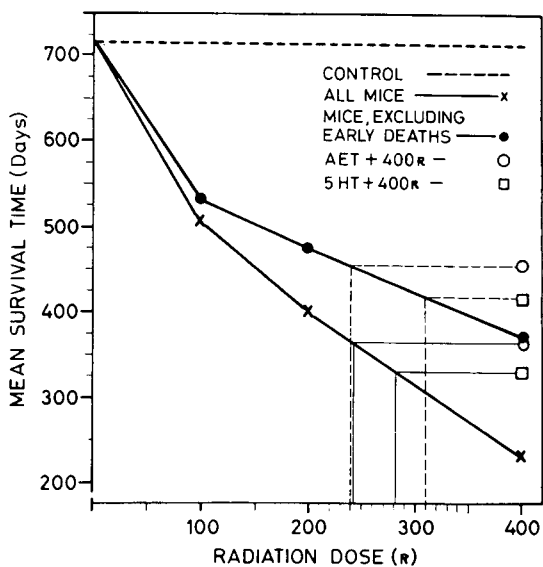


FIG.2. Life-span.

TABLE I. EFFECT ON LIFE-SPAN

Treatment	Animals	Mean life-span (days) ^a	Reduction in life- span (%)	Median life- span (days)
400 R	50	(A) 229 ± 17.7	68	168
		(B) 370 ± 32.6	48	346
200 R	50	(A) 400 ± 24	44	432
		(B) 474 ± 19.4	34	479
100 R	50	(A) 505 ± 19.5	29	526
		(B) 532 ± 15.6	26	528
AET + 400 R	50	(A) 364 ± 23.5	49	367
		(B) 456 ± 19.7	36	460
5-HT + 400 R	25	(A) 330 ± 34.2	54	245
		(B) 417 ± 44	42	398
Control	50	(A) 715 ± 16.7	0	726

^a (A) All mice; (B) Mice, excluding thymic lymphoma and early myeloid leukaemia.

TABLE II. TOTAL NEOPLASM

Treatment	Animals	Total number of neoplasms	Animals with more than one tumour	Mice with neoplasms (%)	Mean life- span (days)
400 R	50	46	5	82	229
200 R	50	44	10	66	400
100 R	50	41	9	60	505
AET + 400 R	50	48	11	74	364
5-HT + 400 R	25	22	4	72	330
Control	50	15	1	28	715

TABLE III. TOTAL NEOPLASM INCIDENCE CORRESPONDING TO MEAN LIFE-SPAN OF MICE EXPOSED TO 400 R

Treatment	Animals	Thymic lymphoma	Myeloid leukaemia	Mammary, ovarian and uterine tumours	Lung tumours	Total neoplasms (per 100 mice)	Mean life-span (days)	Median life-span (days)
400 R	50	31	5	—	—	72	229	168
200 R	50	11	3	—	—	28	400	432
100 R	50	2	1	—	—	6	505	526
AET + 400 R	50	15	6	—	—	42	364	367
5-HT + 400 R	25	7	2	—	3	48	330	245
Control	50	—	—	—	—	0	715	726

It is necessary to point out that in the 400 R group the mean life-span was reduced to 229 days. Compared at 229 days, the total neoplasm incidence (expressed per hundred mice) was 6 in the 100 R, 28 in the 200 R and 72 in the 400 R group. These values indicated a linear dose incidence relationship. The corresponding value was 42 for the AET-protected and 48 for the 5-HT-protected group. A dose reduction factor for total neoplasm induction for AET is about 1.53 and for 5-HT approximately 1.37 (Table III).

3.3. Thymic lymphoma (Fig.3, Table IV)

The frequency and latency of thymic lymphoma and early myeloid leukaemia incidences largely determined the extent of the life-shortening effect observed in the treated groups. Thymic lymphoma death was first observed in the 400 R group at 112 days after treatment. In this group a wave of thymic-lymphoma-connected deaths occurred between 112 and 231 days. During this period, mortality was 72%, including 68% thymic lymphoma. Five of these animals had myeloid leukaemia in association with thymic lymphoma. The rest did not show haemopoietic disease or any major abnormality.

In the 200 R group, deaths of animals with thymic lymphoma were observed during 128 – 244 days. Mortality during the period was 26% and all these were prominently thymic lymphoma cases. Three animals in this group had myeloid leukaemia with thymic lymphoma.

In the 100 R group only three out of 50 mice showed thymic lymphoma. The deaths of these animals occurred at 139, 212 and 248 days after irradiation. The mouse killed on 248 days had myeloid leukaemia also. Beyond 248 days, 92% of the mice survived in the 100 R group as against 100% in the control group.

Thymic lymphoma was absent in the control group.

In the AET group, thymic lymphoma deaths occurred between 134 and 235 days. During the period 32% of the mice died with the disease. Six animals in this group had myeloid leukaemia with thymic lymphoma.

In the 5-HT group, the incidence was 36%. Death of mice with the disease occurred between 154 and 252 days. Two out of nine mice dying with thymic lymphoma had myeloid leukaemia.

The incidence in response to 400 R, 200 R and 100 R shows a linear relationship (Fig.3). This does not exclude the possibility of a curvilinear relationship at doses less than 100 R.

The dose required to produce 50% incidence is about 315 R. It may be observed that the total incidence of thymic lymphoma in the AET + 400 R group corresponds to a dose of 228 R and 5-HT + 400 R to 248 R in the unprotected group (Fig.3). Protection against thymic lymphoma incidences in response to 400 R was 53% by AET and 47% by 5-HT. The dose reduction factor for thymic lymphoma is about 1.75 for AET and 1.61 for 5-HT against 400 R.

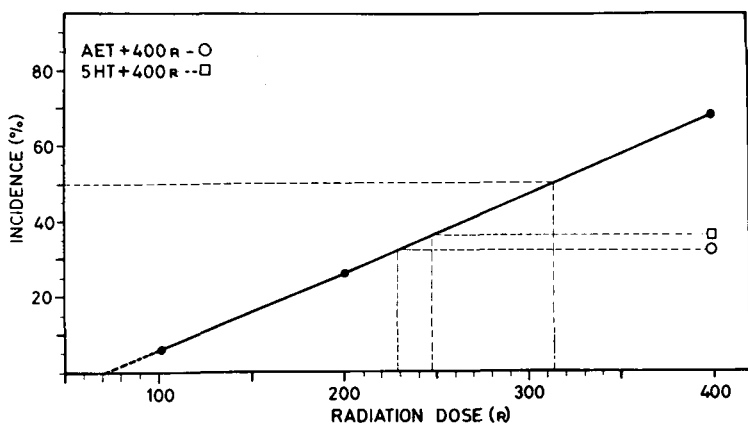


FIG.3. Thymic lymphoma incidence.

TABLE IV. THYMIC LYMPHOMA

Treatment	Animals	Incidence (%)	Mean time at death (days)	Median time at death (days)
400 R	50	68	163 ± 5.6	152.5
200 R	50	26	190 ± 13.6	191
100 R	50	6	200 ± 32	—
AET + 400 R	50	32	167 ± 7.2	161.5
5-HT + 400 R	25	36	200 ± 10.5	194
Control	50	0	—	—

3.4. Myeloid leukaemia (Fig.4)

This disease occurred both early and late in the treated mice. The total incidence was 10% in the 400 R, 18% in the 200 R, 12% in the 100 R, 18% in the AET + 400 R, 8% in the 5-HT + 400 R and 0% in the control group. In the 400 R group and also in the 5-HT + 400 R group all the animals developing myeloid leukaemia were associated with thymic lymphoma. In the 200 R group three out of nine and in the 100 R group one out of six animals dying of myeloid leukaemia had thymic lymphoma. In the AET + 400 R group six out of nine mice had thymic lymphoma as well as myeloid leukaemia.

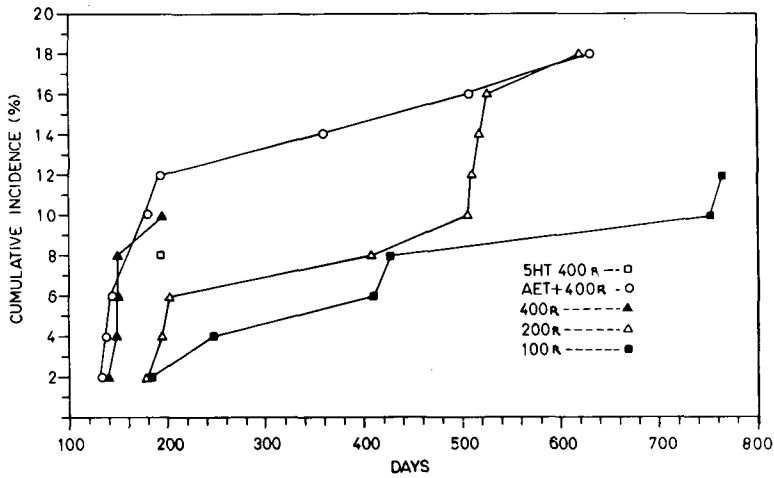


FIG.4. Myeloid leukaemia incidence.

The results of the present study seem to indicate two time periods for incidence of myeloid leukaemia, one occurring fairly early after exposure (< 200 days) and the other towards the end of the life-span. This phenomenon appears to be dose dependent. The animals given 400 R or 5-HT + 400 R and AET + 400 R, all showed an early appearance of myeloid leukaemia within 200 days after exposure. However, the survivors beyond this period in the 400 R and 5-HT + 400 R groups did not develop myeloid leukaemia. The survivors of the AET + 400 R group continued to show myeloid leukaemia. In the 200 R and 100 R groups the incidence of myeloid leukaemia in the early period was low, and the maximum incidence was seen in the later period (> 400 days). The number of animals in the protected and 400 R unprotected groups developing myeloid leukaemia is too small to measure any radioprotective effect of AET or 5-HT.

3.5. Mammary tumour (Fig.5, Table V)

In the 400 R group none of the mice showed the tumour, probably because of the marked reduction in the life-span of these animals.

In the AET + 400 R and in the 200 R group, breast cancer was first detected at 9 to 10 months after exposure, and the cumulative incidences were 10 and 6% respectively.

In the 100 R group mammary tumours developed after approximately 19 months following exposure. Seven out of 21 (33%) animals died with

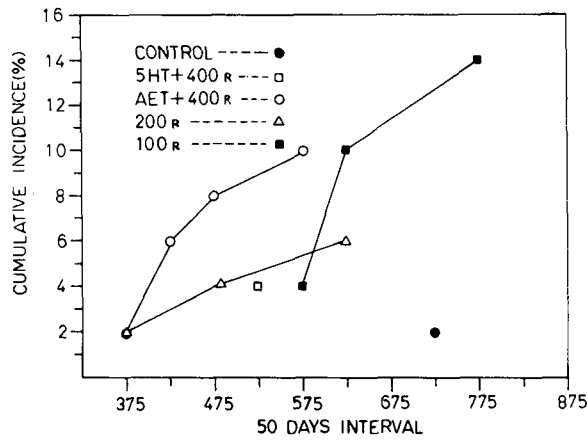


FIG.5. Mammary tumour incidence.

TABLE V. MAMMARY TUMOUR

Treatment	Animals	Incidence (%)	Mean time at death of mice showing mammary tumour (days)	Interval during which death of mice with mammary tumour occurred (days)
400 R	50	0	—	—
200 R	50	6	495 ± 65.8	397 — 620
100 R	50	14	645 ± 30.4	562 — 765
AET + 400 R	50	10	447 ± 32.5	351 — 554
5-HT + 400 R	25	4	504	504
Control	50	2	773	773

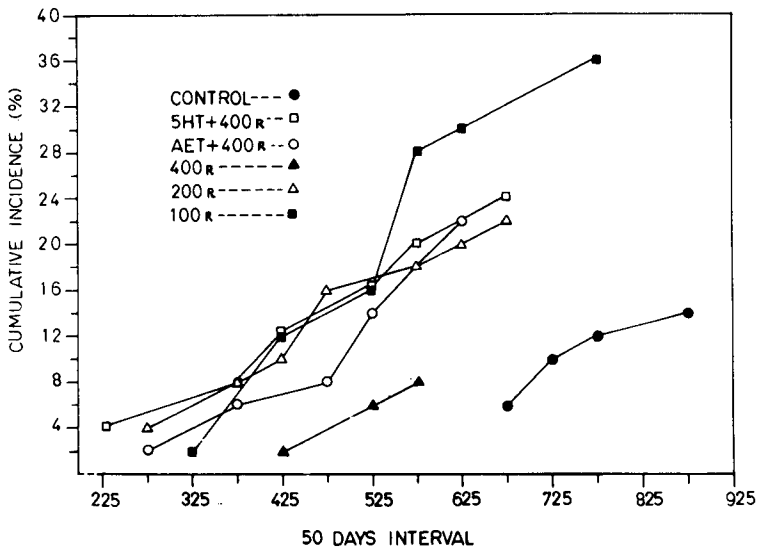


FIG. 6. Ovarian tumour incidence.

mammary tumours. During the same period only one out of 44 animals in the control group died with mammary tumour. The differences are statistically significant.

No clear-cut protection effect of AET and 5-HT could be established because of the small number of animals surviving at this time period. A shift in the latency period as a function of radiation dose is apparent. There is also a suggestion that the mammary tissue is highly sensitive to tumour induction, and if there were no great change in the life-span expectancy, the cumulative incidence would be higher at lower doses.

The main histological types were adenofibroma and adenocarcinoma. Epithelial cells usually formed strings or a network within a solid mass of the tissue. In some cases, they were found to be rich in stroma and surrounded tubular spaces. Cells with little stroma were also observed in some tumours.

3.6. Ovarian tumour (Fig. 6, Table VI)

Most of the ovarian tumours developed in animals surviving beyond 400 days of exposure. For purposes of making comparisons, the tumour incidence at death between 400 and 565 days in the irradiated and control groups has been computed on the basis of the number of survivors at this time period.

TABLE VI. OVARIAN TUMOUR

Treatment	Animals	Mice with ovarian tumours (%)	Mean time at death of mice with ovarian tumour (days)	Interval during which ovarian tumours were detected (days)	Percentage of mice surviving at the beginning of the interval	Percentage incidence in survivors between 400 – 565 days
400 R	50	8	499 ± 35.1	400 – 565	14	57
200 R	50	22	473 ± 36.1	261 – 662	74	30
100 R	50	36	541 ± 28	301 – 765	92	33
AET + 400 R	50	22	493 ± 31.4	291 – 630	64	40
5-HT + 400 R	25	24	456 ± 62	238 – 662	56	60
Control	50	14	737 ± 26	669 – 865	78	0

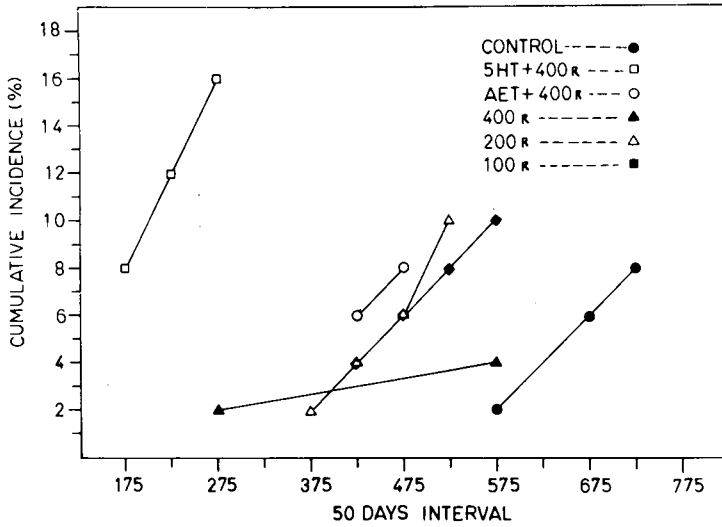


FIG. 7. Lung tumour incidence.

TABLE VII. LUNG TUMOUR

Treatment	Animals	Incidence (%)	Mean time at death of mice with lung tumour (days)	Interval during which death of mice with lung tumour occurred (days)
400 R	50	4	428	291 – 565
200 R	50	10	460 ± 29	356 – 518
100 R	50	10	483 ± 27.9	418 – 567
AET + 400 R	50	8	442 ± 12.5	411 – 472
5-HT + 400 R	25	16	212 ± 22.5	169 – 274
Control	50	8	666 ± 38	565 – 747

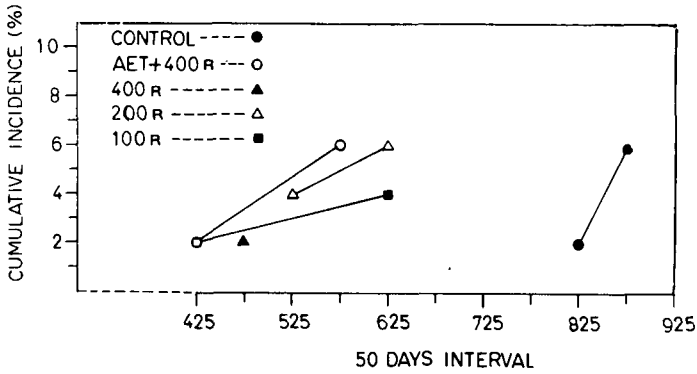


FIG. 8. Uterine tumour incidence.

TABLE VIII. UTERINE TUMOUR

Treatment	Animals	Incidence (%)	Mean time at death of mice with uterine tumour (days)	Interval during which death of mice with uterine tumour occurred (days)
400 R	50	2	468	—
200 R	50	6	550 ± 35.2	506 – 620
100 R	50	4	525	434 – 615
AET + 400 R	50	6	506 ± 50.2	405 – 557
5-HT + 400 R	25	0	—	—
Control	50	6	855 ± 25.4	807 – 894

Whereas there were no ovarian tumours in the control group at this time period, 33% of the animals surviving in the 100 R group, 30% in the 200 R group and 57% in the 400 R group died with ovarian tumours. A dose-effect relationship is evident. Among the survivors of the group given AET + 400 R, 40%, and in the 5-HT + 400 R group, 60% of the animals developed ovarian tumours. The data are not statistically significant to demonstrate a radiation protection of 5-HT or AET on ovarian tumour incidence.

If one were to compute the cumulative incidence of ovarian tumours, which appears to be related to the life-span, the incidence in the 100 R group is about 2.5 times greater than in the control group.

Solid ovarian tumours were smooth and spherical. They varied in size. Granulosa-cell tumours and luteomas were the ones most commonly observed. Adenofibromas and haemangiomas were also seen.

3.7. Lung tumour (Fig.7, Table VII)

In the 5-HT group, the total incidence was 16% as against 8% in the AET and the control group. All the lung tumours in the 5-HT group were observed very early between 169 and 274 days after treatment. Forty-four per cent of the mice survived beyond this period. However, none of them showed lung tumour incidence. Thus in the 5-HT group, the delayed lung-tumour response observed in each of the five groups was absent. These observations indicate that the combined effect of 5-HT and X-irradiation treatments could lead to an early appearance and a higher incidence of pulmonary tumours. Early alveolar cell proliferation and lung tumour incidence in mice following urethan and X-ray treatment [13], modification of urethan lung tumour incidence by cortisone and low X-ray doses [14], and the dose-incidence response for radiation-induced lung tumours [15] in mice were reported.

Large adenomas invading the bronchioles were commonly observed.

3.8. Uterine tumour (Fig.8, Table VIII)

In spite of early mortality and life-span reduction, the uterine tumour incidence in the AET and 200 R group was the same as in the control. This tumour was not found in the 5-HT group.

Uterine tumours varied in size, shape and location. They were seen mostly in one horn only. Generally diffuse growths showing nodular appearance were found.

Sarcomas, fibroids and polyps were the histotypes observed.

4. ASPECTS OF PROTECTION BY AET AND 5-HT

A more or less direct dose-reducing effect is seen in the results of AET protection. 5-HT protection against 400 R prevailed over the late myeloid leukaemia, mammary and uterine tumours. 5-HT being a neurohormone, radiation effects concerned with the neuroendocrinal system are expected to be modified as a result of 5-HT treatment.

In parallel studies the following observations were made.

- (a) 5-HT highly protected against radiation sterility in adult female Swiss mice whereas AET was ineffective. Against low doses 5-HT showed protection even when it was injected soon after irradiation. This observation suggests that 5-HT increased the chances of repair in cell types concerned with these effects.
- (b) In adult male mice, both AET and 5-HT protected all the post-meiotic stages of spermatogenesis. Unlike AET, 5-HT protected spermatogonia, and thereby avoided a stage of temporary sterility observed in unprotected and AET-protected groups.

The natural presence of 5-HT in the various cell types could be concerned with resistance to radiogenic diseases.

5. SUMMARY

The radioprotective effectiveness of AET and 5-HT on life-span shortening was the same as observed for short-term survival.

Though the total numbers of neoplasms in the irradiated, and in the radioprotected and irradiated groups were not significantly different, the tumour incidences were higher than in the control group. Neoplasm incidences in unprotected groups compared at mean life-span of mice exposed to 400 R indicate a linear dose-response relationship. Both AET and 5-HT were a protection against tumours.

Thymic lymphoma incidences indicate a linear dose-response relationship and significant protection with AET and 5-HT.

Response to myeloid leukaemia showed two time periods of incidence. The late response was absent in the 400 R and 5-HT + 400 R groups. The data are not sufficient to measure any radioprotective effect of AET or 5-HT.

Mammary tumour incidence in the 100 R, 200 R and AET + 400 R groups was significantly higher than the level of spontaneous incidence. Mammary tissue in this strain is found to be highly sensitive to tumour induction.

An early appearance and higher incidence of lung tumours in the 5-HT + 400 R group seem to indicate a sensitization effect of 5-HT on pulmonary neoplasm induction with radiation.

A shift in the latency period as a function of radiation dose is apparent with the neoplasms observed.

ACKNOWLEDGEMENTS

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DISCUSSION

C. STREFFER (*Chairman*): You mentioned that you also obtain an effect with 5-hydroxytryptamine administered shortly after irradiation. You

are certainly aware that 5-HT is ineffective under these conditions on survival 30 days after irradiation. Have you any explanation for these differences?

J. PHILIP: I am glad that you raised this important question. The protective effect scored in our experiments was against small doses of whole-body X-irradiation, and was observed in female mice given the minimum dose of radiation required to produce sterility or doses less than those which considerably reduce the litter size compared with untreated controls. The protective effect can be seen if the irradiated females are allowed to mate at intervals following irradiation and the reproductive performance is evaluated. The effect, I think, is probably mediated through the neuroendocrinal system. Some 'repair' of radiation damage at the cellular level is probable. I should mention that a dose of 50 R is a sterility-inducing dose in this strain of mice. At LD₅₀ (30 days), since the doses involved are very high, we fail to recognize the small protective effects of 5-HT. Against lethal and supralethal levels of exposure, the protective effect is diluted to such an extent that it is no longer detectable.

K.H. CHADWICK: In the dose relationship for the induction of thymic lymphoma you had a very good straight line, but I noted that the line did not go through the origin. I wonder if it really is such a good straight line, or whether it is curved. Are you planning to do any more dose points to determine the true shape of this curve?

J. PHILIP: We have at present only three points (against 400, 200 and 100 R) to show the dose relationship for the induction of thymic lymphoma. We hope to obtain more data in this regard. The percentage incidence of thymic lymphoma was 68% in the 400 R, 26% in the 200 R and 6% in the 100 R group as against 0% in the control group. These values indicate a linear dose response, as shown in the graph. The graph is shown to indicate the result of AET and 5-HT protection against 400 R. However, as I mentioned in the penultimate paragraph of Section 3.3 of the paper, the possibility of a curvilinear relationship at doses less than 100 R is not to be ruled out.

E. RIKLIS: Our experience with polyamines such as spermine and cadaverine is that they have a protective effect on V-79 Chinese hamster cells in tissue culture, but at the same time they cause a considerable sensitization to hyperthermia (41.5°C) (Ben-Hur and Riklis, in press). Thus it is necessary to take temperature into account in protection studies.

J. PHILIP: We are aware of the influence of hypothermia and hyperthermia on the end effects in the context of chemical protection and sensitization. I should mention that the animal room temperature was maintained at 22° – 25°C.

CHEMICAL PROTECTION AGAINST LIFE SHORTENING AND CAUSES OF DEATH AFTER A SINGLE OR FRACTIONATED WHOLE-BODY EXPOSURE OF MICE TO IONIZING RADIATION

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Abstract

CHEMICAL PROTECTION AGAINST LIFE SHORTENING AND CAUSES OF DEATH
AFTER A SINGLE OR FRACTIONATED WHOLE-BODY EXPOSURE OF MICE TO
IONIZING RADIATION.

The causes of death and the effect of radioprotection were studied in BALB/c and C57B1 mice exposed to a single dose of X-rays. The causes of death were classified among 12 different diseases and evaluated for competing risks. The life-shortening effect in BALB/c mice depends linearly on the dose, and dose-reduction factors of 1.3 for AET and 2.1 for a protector mixture are obtained. Radiation-induced life shortening is caused by an induction of specific diseases, mainly thymic lymphoma, myeloid leukaemia, glomerulosclerosis and non-cancerous lung lesions. The frequency of lung carcinoma in BALB/c mice decreases after X-ray doses of more than 350 R but its latency period shortens. In BALB/c mice protection is most effective against thymic lymphoma, but is also discernible for myeloid leukaemia and glomerulosclerosis. The protection in C57B1 mice resembles that in the BALB/c strain. Thus, protection is effective against thymic lymphoma (also against liver adenomas, all carcinomas and myeloid leukaemia) as well as against glomerulosclerosis. In another experimental series, mice were subjected to fractionated irradiation at various doses and time intervals until death. Controls and AET-protected mice die when an accumulated dose of about 2 kR is attained; mixture-protected mice can sustain up to 4 kR almost independently of the size and interval of fractions when one corrects for acute lethality. Death is caused by bone marrow failure after short times, and by fibrosis at later periods.

INTRODUCTION

It is still debated whether life shortening by ionizing irradiation is due to an earlier incidence of all diseases and thus to a process of accelerated or precocious aging, or whether it is caused by an induction of specific diseases such as malignant tumours and nephrosclerosis [1, 2].

TABLE I. TIME OF ADMINISTRATION AND DOSES (FOR FIRST INJECTION) OF CHEMICAL COMPOUNDS USED

Treatment	Amount given (mg)	Route of administration	Time of treatment (min before irradiation)
AET (2- β -aminoethylisothiuronium-Br-HBr)	8	ip	10
AET	4	ip	10
GSH (glutathione)	25	o	25
5-HT (serotonin-creatinine-sulphate) (5-hydroxytryptamine)	0.75	ip	5
MEA (mercaptoethylamine)	1.75	ip	10
CYST (cysteine)	10	ip	15

The beneficial effects of radioprotectors on acute sequels of a single exposure to ionizing radiation have been well documented [3, 4]. Some controversy exists, however, as to whether radioprotectors are effective also against the late effects of a single exposure and/or under conditions of repeated exposure [5–7]. During the past few years, we have carried out extensive studies on these two problems. The present report summarizes the results obtained. Details have been published elsewhere [3, 4, 8, 9].

MATERIAL AND METHODS

Studies on life shortening after a single exposure

These studies were carried out on male mice of the BALB/c/Cnb strain, 12 weeks old, and on male mice of the C57B1/Cnb strain, 4 and 12 weeks old, weighing about 25–30 g at the time of exposure. A total of 7373 mice was used in these experiments.

The animals were exposed to X-rays ranging from 100 to 2000 R depending on the treatment (250 kV, 100 R/min, HVL 0.7 mm Cu). Before exposure, the mice were treated either with a mixture of radioprotectors [3] (glutathione, cysteine, 2- β -aminoethylisothiuronium (AET), mercaptoethyl, amine (MEA) and 5-hydroxytryptamine) or with AET alone. Other groups served as non-irradiated or irradiated non-protected controls. The times of administration and the doses of the substances given are listed in Table I.

All mice surviving more than 30 days were selected for the long-term study, and were inspected daily for their entire life-span. At death, autopsies were performed and organ samples were taken for histological examination.

The causes (or possible causes) of death were classified among the following 12 groups:

- (1) Thymic lymphoma.
- (2) Non-thymic lymphoma including reticulosarcoma A.
- (3) Reticulosarcoma B.
- (4) Myeloid leukaemia.
- (5) All leukaemias.
- (6) Lung carcinoma.
- (7) Liver tumours (angioma, adenoma and carcinoma).
- (8) All cancers other than leukaemia.
- (9) Sarcoma.
- (10) Glomerulosclerosis.
- (11) Non-cancerous lung lesions (congestion, infection, fibrosis).
- (12) Other causes of death (infection, fibrosis of various organs, unclear causes of death).

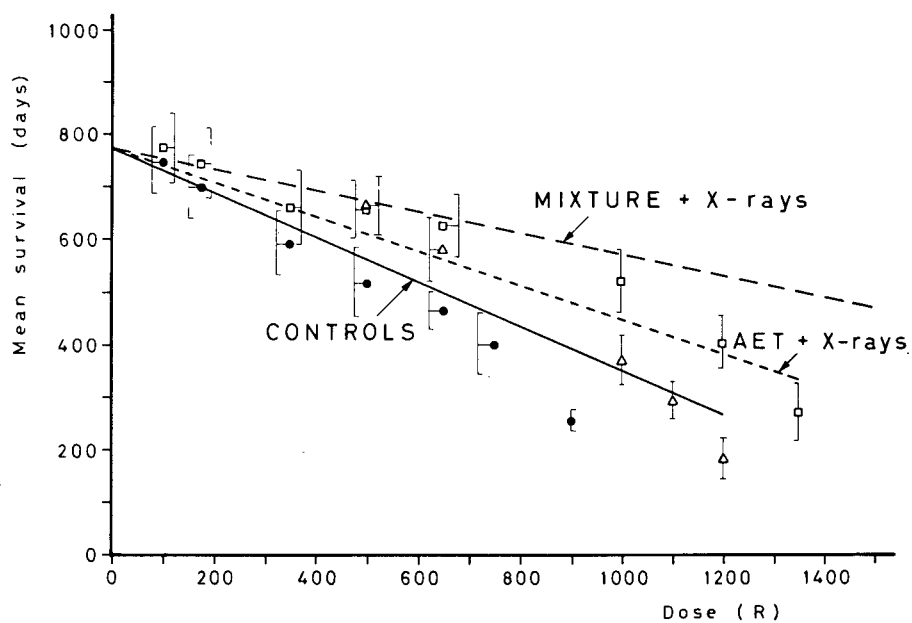


FIG.1. Life-span shortening as function of the dose for non-protected, mixture-protected and AET-protected mice.

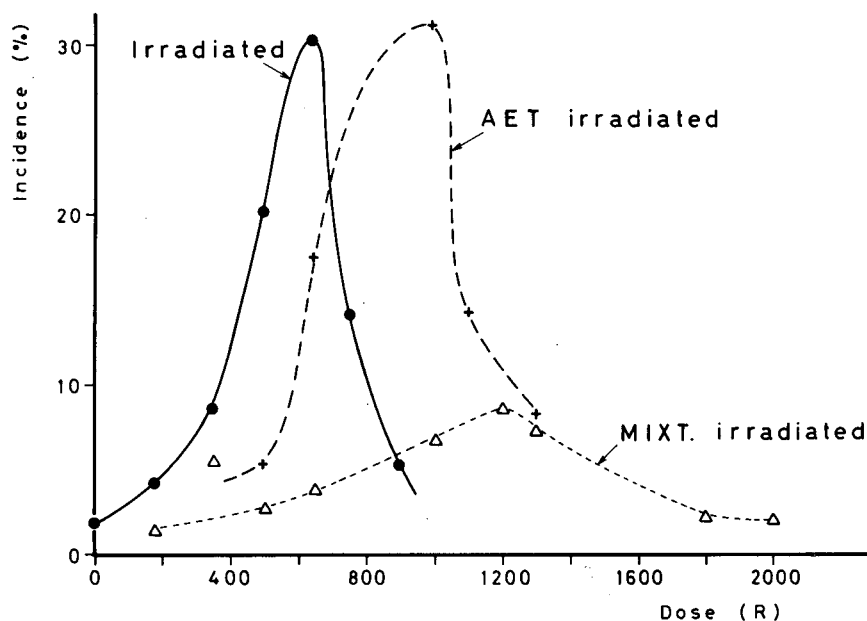


FIG.2. Percentage of thymic lymphoma in BALB/c male mice after X-irradiation and treatment with AET or with a mixture of five chemical protectors.

The causes of death were corrected for competing risks according to the method of Kaplan and Meier [10] as proposed by Hoel and Walburg [11]. The corrected mean ages of death were calculated for each disease using the risk codes 'cause of death' and 'possible cause of death'. The differences between a treated and a control population were tested according to the method of Hoel and Walburg [11]. Differences having a $z < 1.96$ ($p < 5\%$) were considered significant. The numerical calculations were carried out by two computer programs kindly supplied by H.E. Walburg and modified for a Wang 2200 B computer.

Fractionated exposure until death

Male BALB/c/Cnb mice 12 weeks old (a total of 1466 mice) were exposed repeatedly to whole-body exposure at intervals of 7, 15, 30 or 60 days. The irradiation schedule was continued until death of the animal. The conditions of radiation as well as the timing and the dosage of the radioprotectors were the same as for the experiment described above. Since the toxicity of the radioprotectors increases under repeated applications, the doses of AET (in mixture or alone) and of MEA (in mixture) were reduced by about 25% after the first application.

The LD_{50} and the 50% survival times were calculated from the fraction of mice surviving an exposure after correction for 'chemical' death using non-linear regression on the logistic function. In addition, these values were also calculated after correction for the fractions of mice which were expected to die from the acute effects of the last irradiation as calculated from the percentage dying after a single exposure [8].

RESULTS AND DISCUSSION

Life shortening after a single exposure

Life shortening in BALB/c mice from a single exposure follows a linear function of the dose except perhaps at very high doses (Fig.1). Consequently, the life shortening per rad is constant. Pretreatment with a mixture of radioprotectors diminishes significantly the life-shortening factor as does also, to a smaller extent, pretreatment with AET. The dose-reduction factors thus calculated are 2.1 ± 0.23 for the mixture and 1.23 ± 0.047 for AET. These dose-reduction factors are significantly smaller than those attained for acute lethality (2.8 for mixture and 1.7 for AET).

The data on C57B1 mice are not yet as complete as those for BALB mice, but they also show a significant beneficial effect of protection on life shortening. Space does not permit a detailed presentation of the disease spectrum in control, irradiated and irradiated-protected mice of the two strains studied, and only the most prominent features will be mentioned.

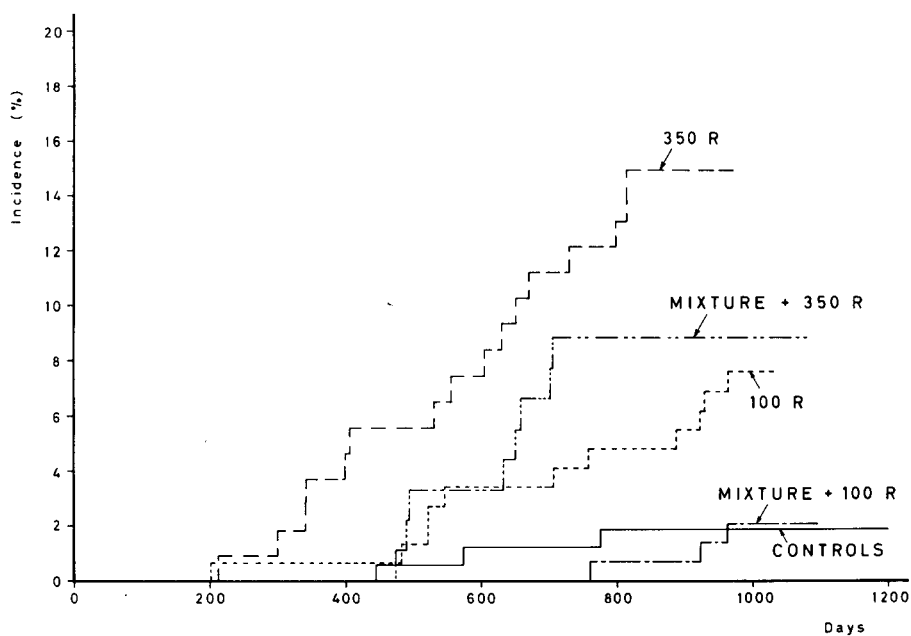


FIG.3. Incidence of myeloid leukaemias in treated and untreated male BALB/c mice with a mixture of five chemical protectors before being exposed to 350 and 500 R.

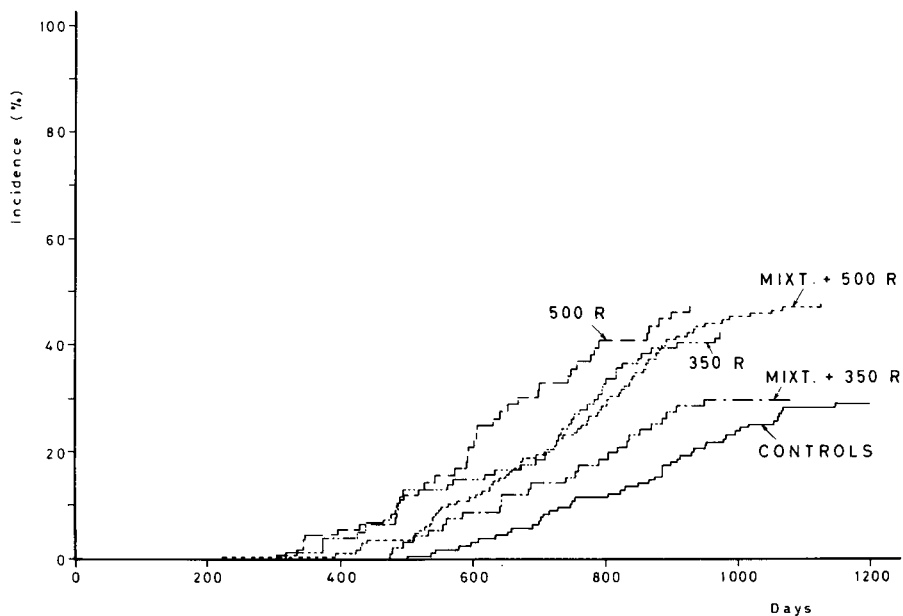


FIG.4. Incidence of glomerulosclerosis in male BALB/c mice treated and not treated with a mixture of chemical protectors before being exposed to 350 and 500 R of X-rays.

Death in non-irradiated BALB/c mice is most often due to lung carcinoma (30%) and non-cancerous lung lesions (24%). Such lung lesions are seen at autopsy even more often (34%), but death cannot always be attributed to them. Non-thymic lymphoma with a frequency of $\pm 10\%$ occupies the third place, followed by glomerulosclerosis ($\pm 6\%$), thymic lymphoma ($\pm 2\%$), reticulosarcoma B ($\pm 2\%$) and myeloid leukaemia ($\pm 2\%$). A few carcinomas are found in the kidney, intestine, skin and adrenals. The latter organ is also the principal site of benign tumours.

The disease spectrum is profoundly altered by irradiation. At low doses the incidence of thymic lymphoma increases markedly reaching a maximum ($\pm 30\%$) at about 650 R and declining at higher doses (Fig.2). The time of appearance (at an age of about 300 days) is, however, not altered by X-irradiation. Myeloid leukaemia is advanced and more frequent after doses from 100 to 500 R (Fig.3) so that a significant increase in all leukaemias is discernible after doses of 350 R or more. On the other hand, the incidence of lung carcinoma diminishes after X-ray doses of more than 350 R, but the latency period is shortened. After doses of 500 R or more, the incidence of glomerulosclerosis (Fig.4) and non-cancerous lung lesions increases, and these diseases occur at an earlier date.

The five diseases mentioned appear to be the main factors determining life-span in irradiated BALB/c mice; for the rest, certain types of tumour increase in frequency, others decrease somewhat but appear earlier (non-thymic lymphoma), a pattern seen also for lung carcinoma, but these causes of death do not greatly contribute to the resulting life-span.

The questions how life-span shortening depends on the dose and how mortality depends on the age of animal (Gompertz plot) have found much attention in earlier studies, and have been variously answered. Thus, some workers found a linear dose dependency [12, 13], others a square one [7, 14] and still others a saturation type function [15]. On the other hand, the Gompertz plots have been interpreted as a sign of accelerated or precocious aging [1] or have given aberrant results [12]. From the analysis of the causes of death it appears that these questions are illusory because they presume a uniform mechanism of death (such as aging due to an equal advancement of all diseases). Indeed, our results as well as those of others demonstrate clearly that there are certain diseases that contribute preferentially to life-span shortening and that, moreover, this disease spectrum depends on the dose range studied. The linear dose relationship found is, therefore, only fortuitous, caused by the overlap of the dose-effect curves of induction of thymic lymphoma and glomerulosclerosis.

Radioprotection alters profoundly the incidence of radiation-induced thymic lymphoma (Fig.2). It has apparently not as much influence on myeloid leukaemia (Fig.3) and on glomerulosclerosis (Figs 4 and 5). Treatment with AET displaces the maximum incidence to a higher dose (1000 R) without altering the height of the maximum, whereas treatment with the mixture diminishes greatly the incidence

	MIXT. + 100 R	MIXT. + 175 R	MIXT. + 350 R	MIXT. + 500 R	MIXT. + 650 R	MIXT. + 1000 R	MIXT. + 1200 R	MIXT. + 1350 R	CONTR.
CONTR			0 4 5 6 (6) 8 (8) (10) (11)	0 10 11 (11)	0 4 5 10 (10) 11	0 1 2 5 10 11	0 1 5 10 11	0 1 5 10 11	
100 R	4 10		0 1 5 (8)	0 10 (11)	0 1 5 10 (10) 11	0 1 2 5 10 11	0 1 2 5 10 11	0 1 2 5 10 11	(10) ↓
175 R	0 ↓ 1 ↓ 4 ↓		10 (10)	0 10 (10) (11)	0 2 5 10	0 1 2 5 10 11	0 1 2 5 10 11	0 1 2 5 10 11	0 ↓ (8) ↓ 11 ↓ (11) ↓
350 R	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (11) ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (10) ↓		0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (11) ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (10) ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 10 11 (11) ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 10 11	0 ↓ 1 ↓ 4 ↓ 5 ↓ 10 11	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (10) ↓ 11 ↓ (11) ↓
500 R	0 ↓ 1 ↓ 2 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ (11) ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (11) ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (11) ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (11) ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (10) ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 10 11	0 ↓ 1 ↓ 4 ↓ 5 ↓ 10 11	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ (10) ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ 11 ↓ (11) ↓
650 R	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 10 11	0 ↓ 1 ↓ 4 ↓ 5 ↓ 10 11	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 10 ↓ 11 ↓
750 R	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓
900 R	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓	0 ↓ 1 ↓ 4 ↓ 5 ↓ 6 ↓ 8 ↓ 10 ↓ 11 ↓

FIG.5. Statistical comparison by the Hoel and Walburg method for normal irradiated and irradiated-mixture-protected BALB/c male mice.

↓ arrow indicates a decreased, no arrow increased, probability for the horizontal group to die compared with the vertical one.

— an underlined decrease code designates significant at the $P < 0.01$ level; not underlined, significant at the $P < 0.05$ level.

○ codes in circles designate possible cause of death; without circles, cause of death.

0 mean survival time.

Numbers refer to the 12 groups listed in the text (Materials and methods).

of thymic lymphoma at all dose levels so that a maximum, when it still exists, would be at doses above 1350 R. Consequently, the residual life-shortening mechanism after protection with a mixture must be due largely to non-cancerous lung lesions and glomerulosclerosis, although to a certain degree protection takes place also with respect to myeloid leukaemia and glomerulosclerosis. The principal difference in effectiveness of AET compared with that of the mixture results from the inability of AET to prevent thymic lymphoma.

It is of interest to compare the data on BALB/c mice with those from a strain of different disease spectrum. Non-irradiated C57B1 mice die most often (42%) from non-cancerous lung lesions and glomerulosclerosis (14%). Lung tumours are rare in this strain, but liver tumours, many of them benign and therefore not a cause of death, occupy a prominent place (18%). Renal changes appear usually as intercapillary nephrosclerosis and are often associated with papillomatous lesions of the penis and prostatitis.

Irradiation changes the disease spectrum of C57B1 mice in a similar manner as in BALB/c mice, increasing mainly thymic lymphoma, and less so all carcinoma, glomerulosclerosis and non-cancerous lung lesions. Protection with a mixture is apparently less effective against thymic lymphoma in C57B1 than in BALB mice, but is apparent also with respect to liver tumours (Fig.6), all carcinomas (Fig.7), and myeloid leukaemia as well as against glomerulosclerosis.

Action of fractionated exposure

These studies aim to determine whether radioprotection is feasible against repeated exposure. To this end, the mice were given fractionated irradiation until they died. Evidently, this design is less suitable to assess quantitatively the extent of repair between the fractions than terminating exposure after a certain number of fractions since some irradiation will be 'wasted'. On the other hand, the procedure utilized approaches more closely potential accidental conditions, and is less demanding in terms of animals required.

The data obtained for the different fractionation schedules are too extensive to be shown here [9]. In short, they indicate that recovery from acute damage had been well completed between fractionation intervals of 30 days or more. A plot of the percentage mortality against accumulated dose for the 30-day intervals shows indeed very little lethality until about 2 kR have been given. Recovery is also well advanced for the 15 and even for the seven days interval provided the single doses did not cause radiation disease. It is thus possible to correct the data for acute lethality. In this way it can be shown that death intervenes when an accumulated dose of about 2 kR is attained for nearly all doses and fractionation intervals investigated. Histological and autoradiographic studies on the bone marrow and the intestine demonstrate that the intestine is restored well, and that

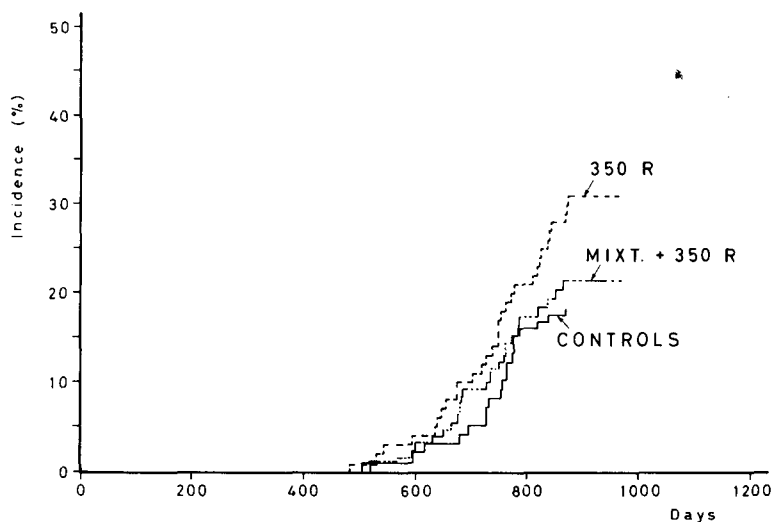


FIG. 6. Incidence of liver tumours in normal C57B1 male mice and in C57B1 mice treated and not treated with a mixture of radioprotectors before being exposed to 350 R of X-rays.

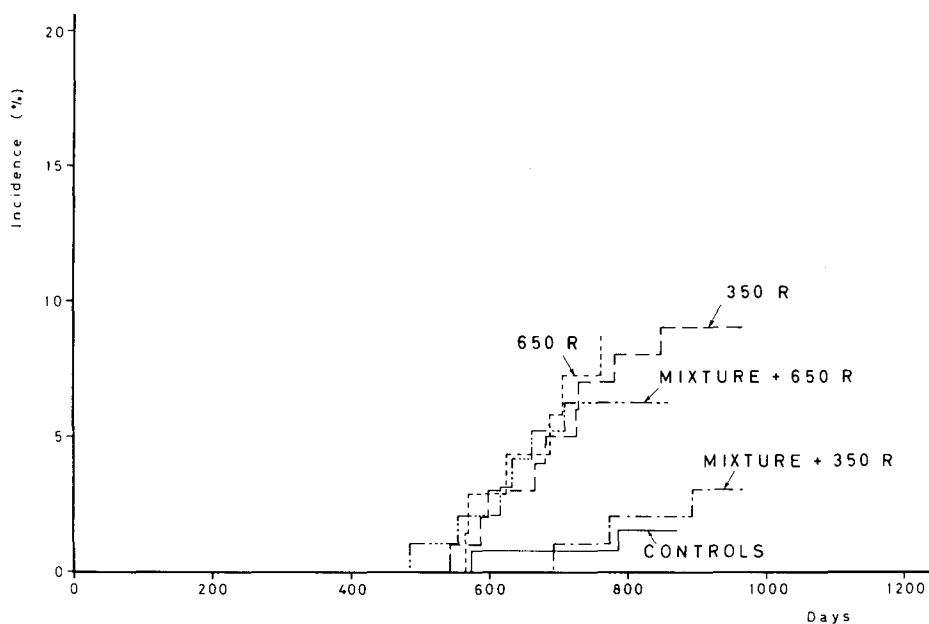


FIG. 7. Incidence of all carcinomas in normal C57B1 mice and in C57B1 mice treated and not treated with a mixture of radioprotectors before being exposed to 350 and 650 R of X-rays.

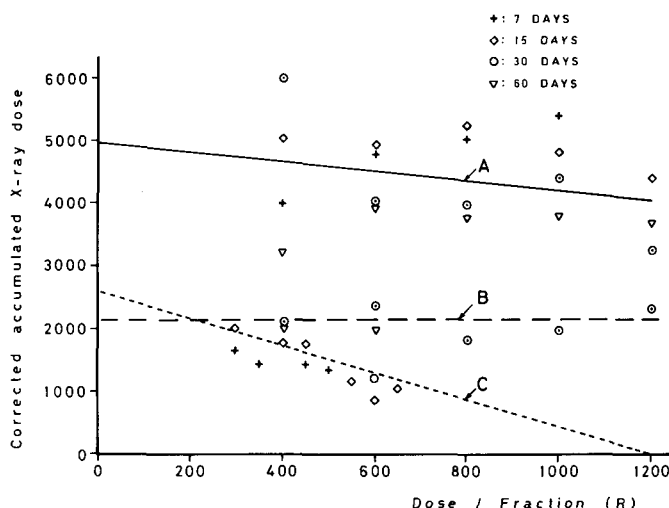


FIG.8. Dependence of corrected accumulated LD_{50} on fraction size for controls (C), AET-protected (B) and mixture-protected mice (A). Extrapolation to dose zero yields 2995 ± 390 , 2123 ± 690 and 4995 ± 503 .

bone marrow recovery is sufficient until the last or the last but one irradiation before death, when an increase in nucleated bone marrow cells forebodes the final exhaustion of the bone marrow. Nevertheless, bone marrow failure appears to be the main cause of death only for the animals dying during the initial six months after initiation of irradiation. Later, fibrosis, particularly of the lung, becomes the principal factor contributing to lethality. The fractionation schedule used attains indeed the dose limit causing late lung damage.

Protection with a mixture is beneficial under repeated exposures; the dose level that can be sustained is raised to 4.5 kR corresponding to a dose reduction factor of about 2–2.5 (Fig.8). This value is, however, still inferior to that observed for acute radiation effects. Protection with AET is much less efficient. Although for a given dose, e.g. 600 R, a significant protection by AET is discernible, this is no longer evident when the curve of LD_{50} against fractionation dose is plotted since the lines for controls and AET-protected mice are not parallel, and intersect at nearly the same LD_{50} for the fractionation doses (Fig.8).

CONCLUSIONS

The data presented demonstrate:

(1) Radiation-induced life shortening after a single exposure is not due to non-specific aging but to the induction of specific diseases, mainly thymic lymphoma, myeloid leukaemia, glomerulosclerosis and non-cancerous lung lesions.

(2) Protection against life shortening by irradiation is feasible, and is particularly efficient with a mixture of chemical protectors (DRF 2.1). Nevertheless, this protection is less than against acute effects of radiation (DRF 2.8).

(3) Protection against life shortening is due mainly to a reduction of thymic lymphoma; it is less marked but still evident for glomerulosclerosis and myeloid leukaemia in both strains and against carcinoma in C57B1 mice.

(4) The dose limit after fractionated exposure at which mice die from irreparable lesions is about 2 kR, but the mechanisms of death depend on the fraction schedules, being bone marrow failure after short periods and fibrosis, particularly of the lung, after longer periods of time.

(5) A mixture protects also against repeated exposure (DRF 2.0–2.5). AET is less or not at all effective.

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DISCUSSION

J. PHILIP: Many of the radioprotective agents are toxic. If two or more protectors are employed, what are the relative amounts of each you would recommend including in the mixture? Is there any principle governing estimation of the net toxicity and the interactions of the protectors in the mixture, particularly when administered into the body? What approach is needed to keep the toxicity low so that we may score high protection?

J.R. MAISIN: It is difficult to answer these questions. The toxicity of a given chemical protective agent can, in fact, vary according to the strain or species of animal concerned. In our system we mixed five radioprotectors. Two of these, cysteine and glutathione, reduce the toxicity of the mixture but are only weakly protective by themselves. We added 5-HT because it does not have the same protection mechanism as the sulphydryl products. We used AET and MEA because at the time we began this experiment they were the most active sulphydryl products, and by using them together we hoped to augment the protective effect obtained.

G.W. BARENDSEN: The effect of the protective agents is presumably dependent on the concentration in various tissues. Is the distribution of AET and of the mixture in the body known, and could differences among tissues cause differences in protection?

J.R. MAISIN: Yes, the degree of protection obtained with different radioprotectors depends on their concentration in the tissues. However, the degree of protection can still vary with the same concentration of a given protector in two different tissues. For instance, the degree of protection obtained with the sulphydryl products depends very much on the reduction they cause in the metabolic activity of the cells. Thus we find that for a given tissue the reduction in metabolic activity occurring after administration of a mixture of radioprotectors is greater than that resulting from administration of one on its own.

C. STREFFER (*Chairman*): Could you also make estimates of the dose-reduction factors from your data on tumour induction, and are they in the same range as for life-span?

J.R. MAISIN: For some types of tumours yes, for others no. In fact the increase in incidence for certain types of tumours after irradiation is too small to enable a dose reduction factor to be derived with any precision. On the other hand, in the case of thymomas the dose-reduction factor obtained in BALB/c mice is greater than two, which is the factor obtained for the life-span.

**GENERAL CONSIDERATIONS,
INCLUDING RISK ESTIMATES**

Session 10

COMPARISON OF THE INCIDENCE AND TIME PATTERNS OF RADIATION-INDUCED SKIN CANCER IN HUMANS AND RATS

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Abstract

COMPARISON OF THE INCIDENCE AND TIME PATTERNS OF RADIATION-INDUCED SKIN CANCER IN HUMANS AND RATS.

Cancer induction in rat skin and human skin are compared following exposure to X-rays. The human data were obtained by follow-up of 2213 children irradiated between 1940 and 1959 for tinea capitis (ringworm) of the scalp. The scalp was irradiated at one session using five fields of 100 kVp X-rays. The scalp dose ranged from 500–800 rads. The rats were irradiated on their dorsal skin with a 1100-rad dose of 30 kVp X-rays. The tumours were predominantly basal cell carcinomas in both species. The proportion of people with tumours as a function of elapsed time since exposure was consistent with a power function with an exponent of 5.4, and had reached 3% or 0.08 tumours per person in the most recent survey (35 years after exposure). Of the 64 tumours observed in human skin, a substantial proportion was on the directly irradiated skin just outside the hair-covered regions of the scalp. So far there are no tumours among the 530 irradiated nonwhites in the study when about eight cases would be expected in a comparable group of irradiated whites. Only four skin tumours have been observed in 1396 control patients. The temporal curve of radiation-induced tumours for human skin could be approximately superimposed on that for rats by contracting the time scale by a factor of 37.1. The temporal response of the two species is approximately proportional to their median life spans.

INTRODUCTION

Extensive use is being made of animal models to assess carcinogenic risks to humans from physical and chemical agents in the environment. Consequently, there is a critical need to obtain as much information as possible on the comparative carcinogenic responses of humans and animals. Extensive studies have been done at the Institute of Environmental Medicine in New York during the past decade on the oncogenic response of rat skin to various types of ionizing radiation and on the pattern of skin tumour occurrence in the scalps of about

TABLE I. A COMPARISON OF IRRADIATED AND NON-IRRADIATED TINEA CAPITIS PATIENTS

	Irradiated	Non-irradiated
Number of subjects	2213	1396
Male (%)	87	79
White (%)	76	75
Mean age at treatment (years)	7.9	7.5
Mean years of schooling	13.0	12.7
Mean interval (years) between treatment and survey	25.5	25.3

2200 patients who were given therapeutic X-ray treatment for tinea capitis (ringworm) as children [1-4]. These data provide an opportunity to compare tumour responses between human and rat in a comparable tissue under comparable conditions of exposure.

MATERIALS AND METHODS

A group of 2213 persons who were irradiated for tinea capitis and a group of 1396 controls who were treated by some other means for tinea capitis have been located, and surveys have been made of their health status in 1967, 1972 and most recently in 1977. These individuals were treated at the N.Y.U. Hospital Skin and Cancer Unit between the years 1940 and 1959. As indicated in Table I, the irradiated and control groups are closely matched for age at time of treatment, race, years of education and elapsed time since treatment. When a person indicated by questionnaire that a tumour had occurred, the time of occurrence, type of tumour and treatment were ascertained from the patient and appropriate physicians or health officials.

The dosimetry of the so-called Adamson-Kienbock procedure has been extensively documented [5]. The X-ray beam was nominally 100 kVp with only the inherent filtration of the X-ray tube. The head was irradiated in five separate exposures of approximately equal duration as follows: front, back, left, right and top. The entire procedure usually required about one hour. Lead plaques were placed over the eyes and ears for all but the back irradiation, and a lead sheet was placed over the face almost up to the hairline for all but the back irradiation. Averaged doses measured in a phantom were as follows: scalp - 650 rads, brain - 140 rads, eyes - 50 rads, thyroid - 6 rads and internal ear - 70 rads.

Skin tumours were noted in relation to elapsed time since exposure for each person and cumulated by means of standard statistical procedures [6]. The ages of the irradiated and control groups were comparable at the time of treatment.

The rats (males only) were irradiated on their dorsal skin (24 cm²) at eight weeks of age with 30 kVp X-rays. These X-rays penetrated with a half value layer of about 0.5 mm so that the internal organs received virtually no dose. The dose to the skin surface was 1100 rads given at a dose rate of about 300 rads/min in a single exposure. Subsequent to irradiation the rats were observed every six weeks for 60 weeks. Tumours were scored when first observed, but only if they persisted at the time of death or sacrifice. The method of analysis was the same as for the human tumours.

RESULTS AND DISCUSSION

The temporal pattern of skin tumour onset as tumours per person (upper curve) and proportion of people with tumours (lower curve) is shown on log-log co-ordinates in Fig.1. The slope gives the exponent of the best-fitting power function, i.e. $P \sim t^{5.4}$, where P is either response and t is elapsed time since treatment. If the tumours were randomly distributed the two curves should be essentially identical since multiple tumours should be rare for values below 0.1 per person. The separation of the curves indicates that the tumours were not distributed randomly. In fact, closer inspection revealed four persons having 33 tumours and the remaining 25 individuals having 37 tumours. It should be noted that the temporal trend in the upper curve where the four high responders were disproportionately represented is the same as in the lower curve where the high responders were relatively insignificant. The similarity in the trends indicates that the temporal pattern was unaffected by the sensitivity of the individuals at risk.

Figure 2 shows the spatial distribution of the 64 skin tumours so far detected, excised and confirmed in the irradiated group. About half of these tumours occurred on skin that might have been exposed to ultra-violet light because of lack of hair cover. Such tumours could, of course, have been induced in part by the ultra-violet light. However, the lack of a substantial number of tumours in the controls, who were presumably as likely as the irradiated group to be exposed to ultra-violet light, suggests that the substantial numbers of tumours on the face, ears and neck were produced at least in part by the X-rays.

On the other hand, it is interesting that none of the nonwhite individuals (about 24% of the total) in the irradiated group have yet developed any skin tumours where about eight cases would be expected in a comparable group of

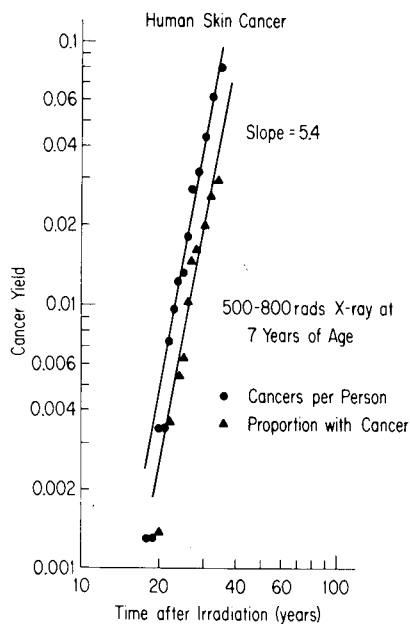


FIG. 1. A log-log plot of skin cancers of the face and scalp among irradiated tinea capitis patients as a function of time after irradiation. The lines shown have a slope of 5.4 which means that yield was proportional to $(\text{time})^{5.4}$. The tumours were exclusively basal cell carcinomas.

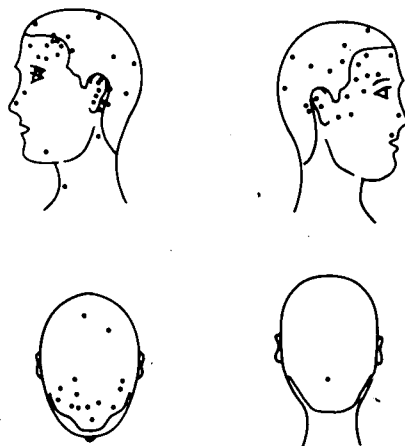


FIG. 2. A scatter diagram showing the location of each of 64 cancers diagnosed and confirmed in the X-irradiated group.

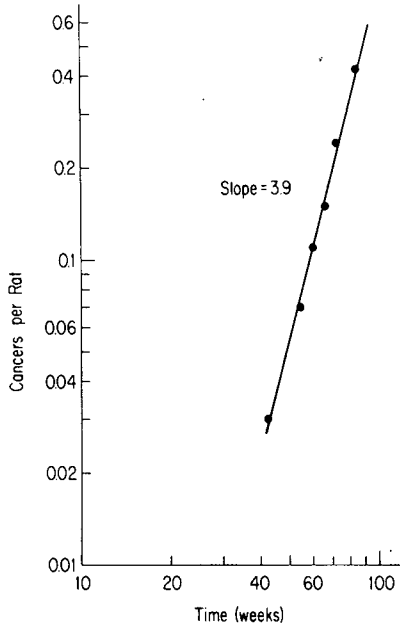


FIG. 3. Cancers per rat as a function of time after exposure to a single 30 kVp X-ray dose of 1100 rads. The tumours were predominantly basal cell carcinomas and sebaceous cell tumours. There were 33 male rats in the group, and about 24 cm² of dorsal skin surface was irradiated on each rat.

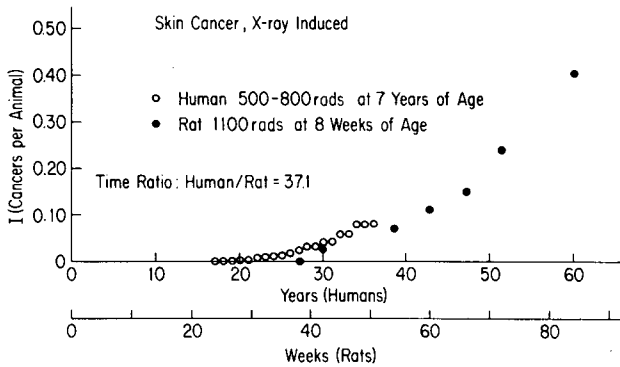


FIG. 4. The cumulative number of cancers per animal for rats and humans are plotted on linear co-ordinates. The dose to human skin was about 500–800 rads and the dose to rat skin was 1100 rads. The time scale was shifted by a factor of 37.1 in order to superimpose approximately the two sets of data.

whites. The lack of tumours among nonwhites suggests that ultra-violet light may be a factor in producing the tumours observed in whites because the skin pigmentation in nonwhites would provide an effective screen against ultra-violet light without significantly affecting the X-ray dose. On the other hand, the lack of tumours among nonwhites may reflect a lower sensitivity of their skin to X-rays.

The temporal pattern of tumour response for the rat skin is shown in Fig.3 on the same log-log co-ordinates as for the human data. The power function exponent was 3.9 for the rat data, but the level of response was necessarily much higher in rats than in humans because of the relatively small number (33) of rats per group.

The same data as in Figs 2 and 3 are shown in Fig.4 on linear co-ordinates with a time scale ratio of 37.1 as indicated. The scale factor was chosen to make the data as consistent as possible in the region where they overlap. If the human data were to continue following a power law with an exponent of 5.4, it would rise above the rat data in a relatively short time; certainly by five years. On the other hand, the human data could bend and follow the rat data at the higher incidence levels. Only a longer follow-up will resolve this issue.

These results suggest that human and rat skin are about equally susceptible to the carcinogenic action of X-ray irradiation under comparable conditions of exposure when allowance is made for the different times for expression of tumour development in the two species. That the time ratio is similar to the ratio of life spans is interesting, and may mean that the life span ratio provides a useful approximation for comparing temporal responses in different species.

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DISCUSSION

J. BERGSMA: In your paper you referred to the application of rather high and penetrating doses to the skin. I wonder if you know anything about Bucky-irradiation, which is apparently a non-penetrating *contact* irradiation applied in intermittent doses for treating superficial dermatological diseases, such as warts, teleangiectasis, angiomas and keloids, and which is claimed not to be harmful. Do you think superficial irradiation of the skin is a safe therapy, without a risk of skin cancer?

F.J. BURNS: Our studies on rats indicate that the tissue most susceptible to radiation carcinogenesis is located in the uppermost 300 μm , which includes the epidermis and hair follicles. We cannot say whether the rat results are applicable to human skin, but certainly no reason comes to mind why the superficial cells in human skin should be resistant to the carcinogenic action of ionizing radiation. There is no doubt that these cells are susceptible to carcinogenesis by ultra-violet light, and this, I believe, should be a signal for caution with ionizing radiation exposure.

J.L. WEEKS: Some years ago we were studying a potential skin carcinogen. In a pilot study we were easily able to raise keratoacanthomata in rats, and in the longer term skin cancers were observed. What was the duration of the UV exposure in your series of rats?

F.J. BURNS: The rats were exposed to single doses and to multiple doses at weekly intervals for 20 weeks. No cancers were observed in these rats after 80 weeks on test, although multiple keratoacanthomata did occur.

RADIATION TUMORIGENESIS IN INBRED LABORATORY ANIMALS AND CANCER RISKS IN IRRADIATED HUMAN POPULATIONS

Two widely different problems

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Abstract

RADIATION TUMORIGENESIS IN INBRED LABORATORY ANIMALS AND CANCER RISKS IN IRRADIATED HUMAN POPULATIONS: TWO WIDELY DIFFERENT PROBLEMS.

The mammal has efficient defence mechanisms against the development of tumours. These mechanisms are successively deteriorated by ionizing radiation when the dose increases beyond certain 'borderline levels'. Consequently, most animal strains demonstrate a bi-phasic dose-tumour relationship with a low-dose limb, the slope of which cannot be distinguished from zero, and a high-dose limb that increases with increasing doses. There are four or five exceptions to this 'rule' but in most of these cases the probable reasons for the deviations are known. Some human tumours as observed in epidemiological investigations do not demonstrate a similar clearly bi-phasic dose response. In all probability, this discrepancy does not reflect a higher susceptibility to radiation-induced tumours in man compared with other mammals. It is rather a consequence of a greater statistical variation in radiosensitivity in heterogeneous human populations than among inbred animals living under standardized conditions. Accordingly, when maximum permissible dose levels are to be determined one should extrapolate from epidemiological data. Furthermore, these extrapolations should be linear if the data do not clearly deviate from a straight line, and if there are no scientific reasons to assume that a threshold exists. This formal method would not produce a biological description of what may happen in the low-dose area but rather an upper risk limit for the population studied. The real low-dose risk cannot be known. For the same pragmatic reason other radiological or non-radiological risks should be determined in the same manner, particularly when risks are to be compared.

INTRODUCTION

From a cellular point of view neoplasia can be considered a dys-differentiation. In this sense the occurrence of cancer in multicellular organisms is not surprising. During the lifetime of man — from the fertilized ovum to death — more than 10^{14} cell divisions take place. This is an enormous number (equal to about 10000 times around the equator calculated in millimetres). The surprising thing

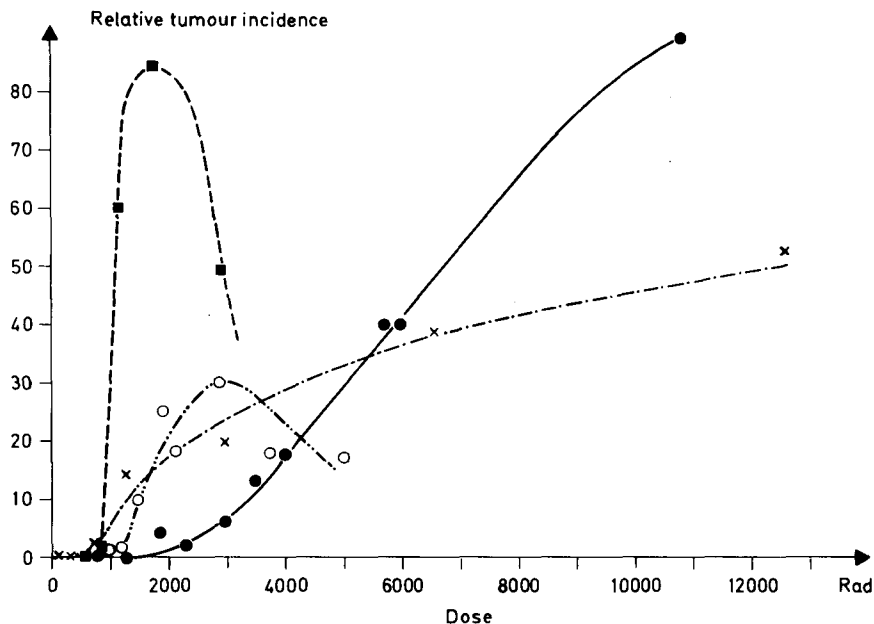


FIG.1. Tumour frequency as a function of dose.

- ^{90}Sr -induced osteosarcomas in CBA-mice [1].
- Kidney tumours in rats induced by X-rays [2].
- Skin tumours in rats induced with electrons [3].
- x---x Bone tumours in man from incorporated ^{226}Ra [4].

is rather the fact that cancer is such a relatively rare disease or, in other words, that the very process of differentiation is such an exact mechanism. Obviously, many 'mistakes' must occur during all these divisions and steps of differentiation which, if not lethal to the cells, would lead to tumour formation provided that the cells have a chance to proliferate. This is, however, obviously seldom the case. The mammalian organism seems to have an extremely good defence capacity against tumour development.

Some years ago many of us believed that these defence mechanisms could be identified as an immunological surveillance by which the cancer cells were rapidly and efficiently eliminated. Although the immunology evidently plays an important role in regulating the tumour growth, it is today generally agreed that it does not have that decisive or clear-cut significance in tumorigenesis. Instead, some recent experimental findings seem to lead us back to Peyton Rous's suggestion that we are bearing a great number of latent tumour cells.

RADIATION-INDUCED TUMOURS

Ionizing radiation can transform normal cells to a latent tumour stage but it can also facilitate the growth of such transformed cells, particularly at higher doses. The latter effect is probably a result of radiation damage to the anti-tumour defence. It is thus not surprising that the experimental dose-tumour relationship often indicates a bi-phasic course with a low-dose limb, the slope of which cannot be distinguished from zero and a high-dose limb that increases with the dose (see Fig.1). There are four or five exceptions to this 'rule' but in most of these exceptional cases we know the probable reasons for the deviations. Unfortunately, the 'exceptional cases' have been so extensively reported and discussed that people sometimes believe that they represent the normal animal tumour response to radiation. This is not the case.

In contrast with the experience from animal experiments some epidemiological investigations do not demonstrate a bi-phasic dose-effect relationship in man. However, this is probably not an indication that man is more radiosensitive with regard to tumours than other mammals, but rather that a human population represents a wider variation in constitutional and way-of-life conditioned susceptibility to radiation-induced tumours than inbred laboratory animals living under standardized conditions.

Figure 2 shows what happens with an integrated probability (gaussian) curve when the standard deviation (σ) is increased. The values to the left of the 50% incidence increase with increasing σ and the curves become increasingly 'linear'. It seems to be a reasonable conclusion that similar differences prevail in comparisons between the effects of a certain agent on inbred laboratory animals and those on heterozygotic populations with highly different individual habits of living (cf. Fig.3). The dose-effect curves obtained from homozygotic animals can be considered as reflecting the true biological effect (the animals may be considered almost 'identical detectors' with regard to the effects of the agent). The corresponding curves obtained from epidemiological investigations are to a greater extent measures of the individual variations in sensitivity to the agents under study.

RISK EVALUATION

When risks are to be estimated the data should, if possible, be taken from epidemiological studies irrespective of the nature of the acting agent. Otherwise, particularly sensitive fractions of a population may be overlooked and we could expect annoying surprises in the future.

Low-dose effects of the order of 10^{-4} or 10^{-5} cannot be scientifically analysed or verified. This statement is something like an uncertainty principle in experimental biology, and has been emphasized in most international reports

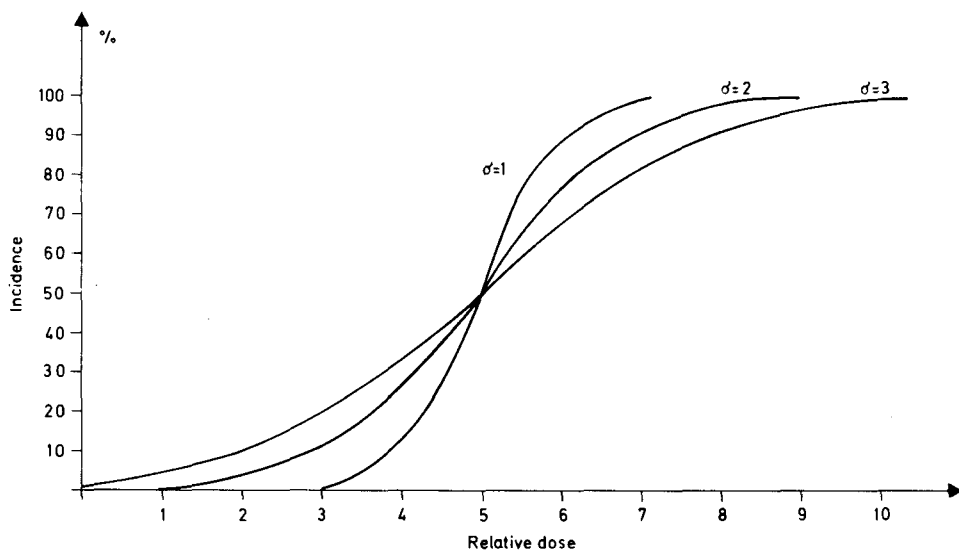


FIG.2. Integrated gaussian curves with three different standard deviations, illustrating how dose-effect curves change when the variation in radiosensitivity increases in irradiated populations.

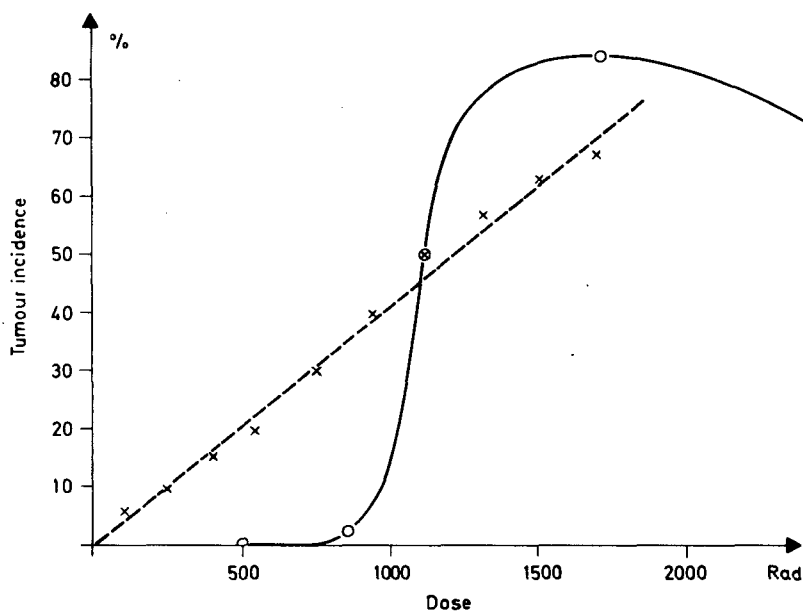


FIG.3. Kidney tumours in X-rayed rats (from Fig.1). The crosses are obtained by increasing the 'standard deviation' six times. As shown by the broken line, these crosses lie close to a straight line through origin.

dealing with radiological cancer risks [5, 6]. Despite this 'biological' statement it is often still claimed in the 'physical' discussions in these reports that there is a proportionality between small dose increments (in the low-dose area) and the corresponding increments of risk. If we consider the hazard to an individual, a prerequisite of this hypothesis is the absence of defence mechanisms that can be stimulated by small dose increments. If anti-tumour homeostasis were of the immunological type the hypothesis would certainly be wrong. Moreover, in radiation protection of human populations the hypothesis is irrelevant. If a certain dose would imply a certain risk to Mr. Brown, twice the dose cannot be presumed to imply twice the risk to Mr. Smith. Mr. Brown and Mr. Smith may have quite different susceptibilities to a particular type of damage.

It is equally wrong to derive mathematical expressions from dose-effect relationships observed in laboratory experiments and apply them to predictions for human populations.

In determining maximum permissible dose levels to man we have to extrapolate epidemiological data which is usually carried out by linear extrapolations from high-dose data. In my opinion this is a pragmatic and relevant method in cases where no thresholds can be verified or scientifically motivated. The method would give an upper risk limit for the population under study. As pointed out by Morgan [7], other populations could have different 'radiosensitivity spectra' giving steeper (or less steep) dose-effect curves. As we know very little about the factors determining the course of these curves it is impossible to predict such relationships.

From experimental radiobiology we know that radiation carcinogenesis is greatly dependent on external and internal factors. For example, it is possible, merely by changing the growth rate of the thyroid gland in the CBA-mouse, to decide whether a certain ^{131}I -dose will result in thyroid tumours or not, whether the tumours will be benign or malignant, and even to some extent the degree of anaplasia.

COMPARISON OF RISKS

The formal, linear risk evaluation can, of course, be extended far beyond radiological cancer hazards. If risks are to be compared the criteria of estimation must obviously be identical. Irrespective of the type of agent and kind of damage we can use the same argument: as long as no thresholds can be found or scientifically motivated we can determine an upper risk limit by linear extrapolations from epidemiological data. This will enable us to compare risks. Figure 4 shows the excess mortalities in Oslo, London and New York as functions of the sulphur dioxide concentration in the air. The sulphur dioxide is, of course, only an 'index substance' representing a general air pollution. Moreover, the victims were

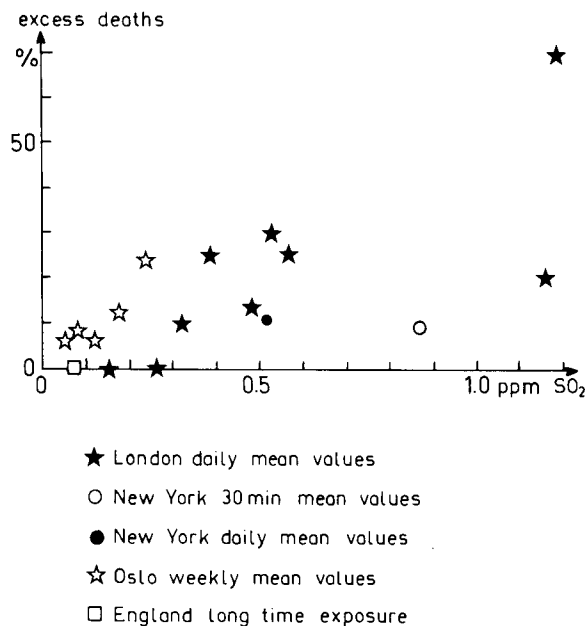


FIG.4. Overmortalities in London, New York, and Oslo as functions of the sulphur concentration in air [8].

chiefly persons who already suffered from various lung-heart diseases. Finally, it must be assumed that the exposure conditions in the three cities were different in many ways. The short exposure in New York gave a smaller figure than the daily mean values which, in turn, lie below the data from Oslo based on weekly means. This suggests an exposure time dependence. The material is, however, too limited to permit conclusions in this respect. Nevertheless, the figure illustrates what will happen in the three cities. As long as we cannot guarantee that these situations will not be repeated at other places and occasions, we must accept a risk estimation based on these data. If we neglect the possible exposure time dependence and determine the regression line, we shall find that the maximum permissible air-concentration of SO₂ for long-term exposures in Sweden (0.05 ppm) would imply an excess mortality of 1.5×10^{-2} . The corresponding cancer risk at the MPL of radiation from nuclear plants in Sweden (1 man·rem/MW-year) is about 1.5×10^{-5} . The conclusions can be handed over to those who are discussing the advantages or disadvantages of nuclear power in comparison with fossil power.

CONCLUSIONS

It is impossible to obtain a knowledge of risks that are too small to be scientifically analysed. This implies that the true risks at doses acceptable as 'maximum permissible' to man cannot be determined.

Upper risk limits may be defined on pragmatic grounds by linear extrapolations from epidemiological data provided that no thresholds can be observed or scientifically motivated, and that the populations are sufficiently large to exclude a significant contribution from extremely sensitive persons (hump effects on the dose-effect curve).

Accordingly, a formal regression line cannot reflect a biological reality but only an upper risk limit (and properly speaking only for the population studied). In my opinion, we have, however, no other alternative if we wish to avoid annoying surprises and if we want to compare otherwise incommensurable risks. The lack of epidemiological data, for instance concerning most carcinogenic substances, forces us to accept risk levels (based on experimental studies or from tests carried out on a few volunteers) that are higher than those for exposures to ionizing radiation. It is necessary to keep this in mind when comparing the hazard of ionizing radiation with that of other agents, otherwise one may strain at a gnat and swallow a camel.

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DISCUSSION

P.G. GROER: I agree with your statement in your oral presentation that 'linear' extrapolation is conservative — even too conservative in some cases. But the following question arises: do you know from your methodology — you mentioned statistics — that this is the case?

G. WALINDER: The linear extrapolation of observed epidemiological data is probably conservative, provided that we consider large populations who have not been exposed to some significant agent or factor that might have a synergistic effect on the neoplastic development. For especially sensitive fractions of a human population the dose-tumour curve may be steeper.

D. GRAHN: I should like to make several comments concerning your observations. First, the variance of animal populations can be increased by a factor of 2–3 by genetic manipulation, but this does not change the basic dose-response relationship, as suggested in your analysis. The response rate is dependent on the dose, the life expectancy, age at exposure and in particular on the statistical methods employed. Second, the premise that cancer is a rare disease in man cannot be supported by present-day vital statistics, especially in the western industrial nations. As life expectancy increases, the probability of death from cancer also increases, so that for those populations with a life expectancy of 70+ years, cancer deaths make up 1/5 to 1/4 of all deaths. Incidence rates approach one in two per person per lifetime. Third, plotting the death rate from breast cancer from national averages around the world versus the estimated national dietary intake of fats (as in your oral presentation) gives a false impression of a rather close linear relationship. Such comparisons can be made only after standardization for age and parity, but even those corrections do not allow for racial, cultural or other personal factors that may influence the incidence and death rates.

G. WALINDER: What exactly do you mean when you say, 'The variance of animal populations can be increased by a factor of 2–3 by genetic manipulation'? Increased in what respect? If the standard deviation *with respect to the radio-sensitivity to cancer* is increased, as shown in Fig.2 (e.g. by hereditary or environmental factors), the effect would of course be exactly as is shown in the figure. I have not discussed the possibilities of doing this with a particular strain.

The cancer incidence in man is surprisingly low in view of the fact that more than 10^{14} cell divisions occur during our lifetime and that the processes we call 'differentiation', despite their complexities, are so exact.

The point I was trying to make with my plot of death rate versus fat intake (in my oral presentation) is precisely what you have just stated. I said that it would be a great over-simplification to ascribe the differences in breast cancer mortality to fat consumption. This curve was in fact one of my arguments against the possibility of finding simple solutions to variations in cancer incidence in different populations. The figure does show that breast cancer frequency differs

greatly between different populations. We have to accept that fact and base our evaluations of risks on it without pretending to know the causes.

R.E. LINNEMANN: What fraction of the human population is more sensitive to radiation-induced cancer, and how would we identify this fraction?

G. WALINDER: I wish I could answer that question. In my opinion this is one of the great challenges to radiobiological research, and more attention should be directed towards the significance of external and internal factors for the late radiation effects.

F. DEVIK: There are reports in the literature which indicate that there may be subpopulations with relatively high sensitivity to cancer induction by X-rays — for example, the investigations by Hempelmann and co-workers on thyroid cancer, which showed Jewish girls to be much more at risk, and the work some years ago by Bross and Natarajan, who studied the frequency of malignant diseases in children suffering from other diseases as well.

A COMPETING RISK MODEL FOR REDUCTION IN LIFE EXPECTANCY FROM RADIOGENIC CANCER*

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Abstract

A COMPETING RISK MODEL FOR REDUCTION IN LIFE EXPECTANCY FROM RADIOGENIC CANCER.

Latent radiogenic cancer fatalities from reactor accidents are considered to be more important than early fatalities. However, early fatalities generally result in appreciable life shortening for the affected individual whereas latent cancer fatalities generally result in limited life shortening. In this report a mathematical model is developed to express the reduction in life expectancy from radiogenic cancer as a function of dose received. The model is then used to compare the linear model of latent radiogenic cancer incidence with several nonlinear models that have appeared in the literature.

1. INTRODUCTION

Because of the large number of persons who could potentially receive low doses of radiation as a result of a nuclear reactor accident, the number of delayed fatalities from radiogenic cancers may be larger than the early, or prompt, fatalities [1]. For this reason the radiogenic cancer fatality risk of reactor accidents may be considered as being more important than the early fatality risk. In addition, there exists the temptation to add the radiogenic cancer fatality risk to the early fatality risk for the purpose of comparing reactor accident risks with other risks that society is exposed to, such as automobile accidents, airplane accidents, hurricanes, etc. However, the impact on the individual, and society as a whole, from radiogenic cancer fatalities is significantly different from the impact produced by prompt fatalities. In this report we develop a mathematical model to express the reduction in life expectancy from latent radiogenic cancer as a function of dose received.

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TABLE I. DOSE EFFECTIVENESS FACTORS

rems exposure	at <1 rem/day	at 1-10 rem/day	at >10 rem/day
10	0.2	0.2	0.2
10-25	0.2	0.4	0.4
25-300	0.2	0.4	1.0

The incidence of fatalities from radiogenic cancers has been very extensively studied (see, for example, Refs [1-5]). These fatalities can only be described by a probability function and are dependent on dose parameters and other biological, socio-economic and environmental variables. Because no clinical distinction can be made between 'normally' occurring cancers and radiation-induced cancers, the above studies consider the excess incidence of cancer fatalities for exposed populations.

The basic model used in the above reports for the calculation of radiogenic cancer fatalities assumes that the incidence is a linear function of dose. This so-called 'Linear Hypothesis' has many detractors and has caused much controversy (see, for example, Refs [6-8]). A major concern is one of possible repair mechanisms that result in a nonlinear response to low LET radiation at low doses and dose rates.

Several nonlinear modifications to the linear hypothesis have been presented. In the Reactor Safety Study [1], for example, the linear hypothesis was used to produce an upper bound on incidence. To account for possible repair processes, dose effectiveness factors were used to correct the effect of low doses or low dose rates as multipliers of the risk coefficients (Table I). These dose effectiveness factors represent a step function approximation to a supposedly continuous function.

A model mentioned in the Reactor Safety Study as a possible lower bound on dose response was that of Mays and co-workers [9]. The Mays model assumes that the incidence of radiogenic cancer increases as the square of the dose.

The models for radiogenic cancer fatalities in Refs [1] and [2] further assume a latent period following the radiation exposure in which no excess cancer fatalities occur, followed by a plateau period of uniformly distributed fatalities. The risk of latent cancer fatalities is usually expressed as the incidence rate during the plateau period in terms of cases per million exposed population per year per rem. The total number of cases per million exposed population per rem (cases per million man·rem) is obtained as the yearly incidence rate times the length of the plateau period.

The latent period is assumed in the BEIR model to be independent of dose received. Evidence exists indicating, however, that the latent period tends to decrease with increasing dose (see, for example, Refs [10–13]). Jones and Grendon [13] also propose a model of the latent period decreasing as the cube root of dose,

$$\ell = \ell_0(D_0/D)^{1/3} \quad (1)$$

where ℓ_0 is the known latent period for dose D_0 and ℓ is then the latent period corresponding to dose D .

2. THE MODEL

Several studies of radiogenic cancer effects use excess cancer deaths since it is not possible either to differentiate clinically a radiogenic cancer from other cancers, or to detect if a person who has died of some natural cause would have developed radiogenic cancer. The first problem is then to compute the probability of dying of a radiogenic cancer given that the cancer is incipient. Notationally, we seek $P(CA_i|L_i, d, a)$ where CA_i is the event of dying of latent radiogenic cancer of type i , L_i is the event that the carcinogenic mechanism for cancer of type i is or will be fulfilled as a result of a dose of d rems at the age of a years.

In the usual method for competing risk models, we assume that the competing risks are independent. That is that the time to death of natural causes is independent of the time to death from radiogenic cancer. The probability of surviving x years is then expressed by

$$P(S \geq x|L_i, d, a) = P_\eta(S \geq x|a)P_{L_i}(S \geq x|d, a) \quad (2)$$

where P_η is the probability of surviving normal risks and P_{L_i} is the probability of surviving death due to radiogenic cancer given that the carcinogenic mechanism is or will be fulfilled. If we let X be the time to death, and in the usual manner for competing risk models, $X = \min(X_\eta, X_{L_i})$ where X_η is the time to death from natural causes and X_{L_i} is the time to death from radiogenic cancer of type i , then

$$P(X \geq x, CA_i|L_i, d, a) = P(x \leq X_{L_i} \leq X_\eta|L_i, d, a) \quad (3)$$

From this it follows that

$$P(CA_i|L_i, d, a) = \int_0^\infty P_\eta(S \geq x|a)f_{ia}(x)dx \quad (4)$$

where

$$f_{ia}(x) = \frac{d}{dx} P_{L_i}(S < x|d, a)$$

which is the density function of survival time given L_i , d and a .

By assuming that for radiogenic cancer the time from exposure to death (X_{L_i}) has a uniform distribution with latent period ℓ_{ai} and a plateau period Δ_{ai} , this expression can be reduced to

$$P(X \geq x, CA_i | L_i, d, a) = \begin{cases} P(CA_i | L_i, d, a) & x \leq \ell_{ai} \\ \frac{1}{\Delta_{ai}} \int_x^{\ell_{ai} + \Delta_{ai}} P_\eta(S \geq v|a) dv, & \ell_{ai} < x \leq \ell_{ai} + \Delta_{ai} \\ 0 & x > \ell_{ai} + \Delta_{ai} \end{cases} \quad (5)$$

Let $S(x)$ be the portion of the population surviving normal risks to age x , $S(x) = P_\eta(S \geq x|0)$, then

$$P_\eta(S \geq x|a) = \frac{S(x+a)}{S(a)} \quad x \geq 0$$

This then simplifies to

$$P(CA_i | L_i, d, a) = \frac{1}{S(a)\Delta_{ai}} \int_{\ell_{ai}}^{\ell_{ai} + \Delta_{ai}} S(a+x) dx \quad (6)$$

From the preceding, we can express the probability of the unobservable event L_i as

$$P(L_i | d, a) = \frac{P(CA_i | d, a)}{P(CA_i | L_i, d, a)} \quad (7)$$

where $P(CA_i | d, a)$ can be estimated from excess cancer fatalities. Unfortunately, $P(CA_i | d, a)$ is not tabulated for every age a , but rather it is $P(CA_i | d, a \in A_j)$ that is available, for example [1], where

j	1	2	3	4
A _j	in utero	[0, 10)	[10, 20)	[20, ∞)

It is easily shown that

$$P(CA_i|d, a \in A_j) = \frac{\int_{a \in A_j} P(CA_i|d, a) \Pi(a) da}{\int_{a \in A_j} \Pi(a) da} \quad (8)$$

where $\Pi(a)$ is the probability density for age in the population. This, however, does not allow us to compute $P(CA_i|d, a)$ for every value of a .

A model for $P(L_i|d, a)$ which allows solving Eq.(8) and which is quite general allowing consistency with the linear hypothesis, the dose effectiveness model, or the quadratic model of Mays and co-workers, is to assume that the processes of dose effect and age decompose, i.e.

$$P(L_i|d, a) = \phi(d) \Psi_i(a) \quad (9)$$

for some functions $\phi(\cdot)$ and $\Psi(\cdot)$. The linear hypothesis, then, is consistent with $\phi(d) = d$, the dose effectiveness model with $\phi(d) = d \times$ dose effectiveness factor, and the quadratic model with $\phi(d) = d^2$. Equation (8) then reduces to

$$P(CA_i|d, a \in A_j) = \frac{\phi(d)}{\pi_j} \int_{A_j} \Psi_i(a) P(CA_i|L_i, d, a) \Pi(a) da \quad (10)$$

where

$$\pi_j = \int_{A_j} \Pi(a) da$$

Since the number of radiogenic cancer fatalities is considerably small relative to the population at risk, the probability distribution on the number of excess cases is well approximated by the Poisson distribution with

$$N \times P(CA_i|d, a \in A_j) = \text{expected number of cases}$$

If we let ρ_{ij} denote the risk factor, the expected number of radiogenic latent cancer fatalities of cancer type i per year from exposure at age $a \in A_j$; let C_{ij} be the dosimetry factor that converts 1 rem total exposure to C_{ij} rems to the site

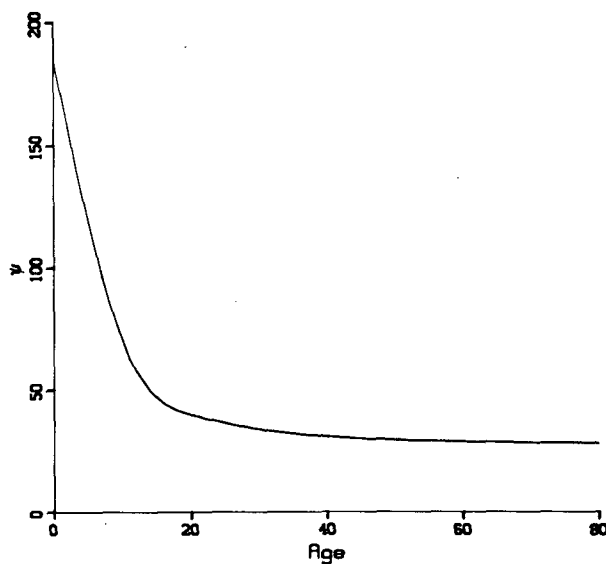


Fig.1. Estimate of Ψ for leukaemia.

at risk for cancer type i and age group A_j , and let Δ_{ij} be the length of the plateau period for age group A_j and cancer type i , then we have by the linear hypothesis

$$P(CA_i|d, a \in A_j) = C_{ij}\rho_{ij}\Delta_{ij} 10^{-6} d \quad (11)$$

Using Eq.(11), several solutions to Eqs (8) and (10) are possible. Figure 1 is a numerical solution of Eq.(10) for $\Psi_i(a)$, for leukaemia, using spline functions. A second solution can be obtained which is more readily computable and quite consistent with the linear hypothesis by assuming that

$$\Psi_i(a) = 10^{-6} K_{ij} \quad \text{for } a \in A_j \quad (12)$$

That is, K_{ij} is a risk factor for the number of radiogenic cancers 'initiated' per million man·rem. Then

$$P(L_i|d, a) = K_{ij} 10^{-6} d \quad \text{for } a \in A_j$$

and

$$K_{ij} = \frac{\pi_j C_{ij}\rho_{ij}\Delta_{ij}}{\int_{a \in A_j} P(CA_i|L_i, d, a)\Pi(a)da} \quad (13)$$

The survival function for the radiation-exposed population, $P_R(S \geq x|d, a)$, is that of a mixture of populations characterized by the events $(L_1, L_2, \dots, L_M, \sim L)$, that is the events that radiation will result in fulfilment of the carcinogenic mechanisms for the various types of latent cancers plus the radiated population in which the carcinogenic mechanisms will not be fulfilled. Because the individual events $(L_i, i = 1, M)$ are rare events, a slight over-estimate of risks is made by assuming them to be mutually exclusive. It then follows that

$$P(\sim L|d, a) = 1 - \sum_{i=1}^M P(L_i|d, a)$$

and

$$\begin{aligned} P_R(S \geq x|d, a) &= P(\sim L|d, a)P_\eta(S \geq x|a) \\ &+ \sum_{i=1}^M P(L_i|d, a)P_{L_i}(S \geq x|d, a)P_\eta(S \geq x|a) \\ &= P_\eta(S \geq x|a) - \sum_{i=1}^M P(L_i|d, a)P_{L_i}(S < x|d, a)P_\eta(S \geq x|a) \end{aligned} \quad (14)$$

The life expectancy of a non-irradiated population is given by

$$E[X|a] = \int_0^{\infty} P_\eta(S \geq x|a) dx \quad (15)$$

and that of a population exposed to d rems at age a is given by

$$E[X|d, a] = \int_0^{\infty} P_R(S \geq x|d, a) dx \quad (16)$$

The reduction in life expectancy can then be expressed as

$$\text{RLE}(d, a) = E[X|a] - E[X|d, a] \quad (17)$$

$$= \sum_{i=1}^M P(L_i|d, a) \int_0^{\infty} P_{L_i}(S < x|d, a) P_{\eta}(S \geq x|a) dx$$

The linear hypothesis model, the dose effectiveness model of NRC and the quadratic model of Mays and co-workers, all assume that ℓ_{ia} and Δ_{ia} are not functions of dose. Consequently, for these three models,

$$P_{L_i}(S < x|d, a) \equiv P_{L_i}(s < x|a)$$

$$\text{and} \quad (18)$$

$$\int_0^{\infty} P_{L_i}(S < x|a) P_{\eta}(S \geq x|a) dx$$

are independent of dose. It then follows that

$$\text{RLE}(d, a) = \phi(d) \sum_{i=1}^M \Psi_i(a) \int_0^{\infty} P_{L_i}(S < x|a) P_{\eta}(s \geq x|a) dx \quad (19)$$

and hence that $\text{RLE}(d, a) \propto \phi(d)$. That is, for the linear model, the reduction in life expectancy is a linear function in dose. Also, assuming $\Psi_i(a) = 10^{-6} K_{ij}$, it follows that

$$\text{RLE}(d, a) = 10^{-6} d \sum_{i=1}^M K_{ij} \int_0^{\infty} P_{L_i}(S < x|a) dx \quad (20)$$

for the linear model.

One last interesting question is that of the average years lost per radiogenic cancer fatality. This calculation is more useful than RLE for use with existing

reactor safety studies such as Ref.[1], as the number of radiogenic cancer fatalities is available output.

If a population of size N_a consisting of people of age a is exposed to d rems, then the Years Lost Per Fatality from type cancer i is

$$\begin{aligned} \text{YLPF}_i(d,a) &= \frac{N_a \text{RLE}_i(d,a)}{N_a P(\text{CA}_i|d,a)} \\ &= \frac{P(L_i|d,a) \int_0^\infty P_{L_i}(S < x|d,a) P_\eta(S \geq x|a) dx}{P(L_i|d,a) P(\text{CA}_i|L_i, d,a)} \\ &= \frac{\int_0^\infty P_{L_i}(S < x|d,a) P_\eta(S \geq x|a) dx}{P(\text{CA}_i|L_i, d,a)} \end{aligned} \quad (21)$$

which is independent of the model used to obtain $P(L_i|d,a)$. Further, if the latent period is not a function of dose, then from Eqs (6) and (18),

$$\text{YLPF}_i(a) = \frac{\int_0^\infty P_{L_i}(S < x|a) P_\eta(S \geq x|a) dx}{P(\text{CA}_i|L_i, a)} \quad (22)$$

is completely independent of dose and can be multiplied by the number of radiogenic cancer fatalities to obtain the total years lost.

3. APPLICATION

The formulas outlined in the previous section have been implemented into a computer program. For illustrative purposes, we shall assume an exposure from ^{137}Cs to allow determination of the dosimetry factors C_{ij} , as the American Physical Society reactor safety study [14] determined ^{137}Cs to be the major cause of predicted cancer deaths.

The age distribution $\Pi(a)$ and the survival function $S(a)$ were obtained from the 1970 vital statistics of the United States of America [15]. In Table II we present the values extracted. Since values of Π and S are needed for all ages (i.e. in one year intervals), a standard method of using splines to interpolate was employed. Also, because it is very important to know S for all older ages (the tables stop at 85), an ideal assumption was made that the population has no one in excess of 100 years of age (this resulted in at most a 0.1% error in the life expectancy values also published in Ref. [15]).

TABLE II. LIFE TABLES [15]

Age interval	S(x) of 100 000 born alive	Stationary population
0-1	100 000	98 189
1-5	97 984	391 133
5-10	97 653	487 712
10-15	97 448	486 793
15-20	97 250	485 022
20-25	96 715	481 825
25-30	96 002	478 310
30-35	95 312	474 602
35-40	94 491	469 475
40-45	93 330	462 599
45-50	91 585	451 806
50-55	88 945	435 607
55-60	85 069	412 091
60-65	79 458	379 204
65-70	71 858	335 334
70-75	61 898	279 788
75-80	49 664	213 104
80-85	35 334	140 305
85 and older	21 077	122 292

The dose-effectiveness model, quadratic model and the cube-root latent period model were all studied for comparison with the linear model. The dose-effectiveness model used the risk factors adjusted by dose as used in Ref.[1] (see Table I). As suggested by Mays and co-workers [9], the total number of radiogenic cancers for the linear and quadratic models are taken to intercept at 1250 rem for leukaemia and 310 rem for all other fatal cancers ($0.004^{-1} \times 5$ and $0.016^{-1} \times 5$, respectively). To be consistent, it was also assumed for the Jones and Grendon model that the latent periods assumed in Refs [1] and [2] apply for $D_0 = 1250$ and 310 rem (see Eq.(1)) for leukaemia and all other fatal cancers, respectively. The risk factors ρ_{ij} (expected cases per million man·rem)

TABLE III. RISK FACTORS FOR CANCER MORTALITY [1]

Type of cancer and age of irradiation	Latency (a)	Plateau (a)	Risk
Leukaemia:			
In utero	0	10	15
0-9.9 years	2	25	2
10+ years	2	25	1
Lung cancer:			
10+ years	15	30	1.3
Gastro-intestinal tract:			
Stomach, 10+ years	15	30	0.6
Rest of gastro-intestinal tract, 10+ years	15	30	0.2
Pancreas, 10+ years	15	30	0.2
Breast cancer, 10+ years	15	30	1.5 ^a
Bone cancer:			
0-19.9 years	10	30	0.4
20+ years	10	30	0.2
All other cancers:			
In utero	0	10	15 ^b
0-9.9 years	15	30	0.6 ^c
10+ years	15	30	1 ^d

^a Includes males and an assumed 50% cure rate.

^b 'All other' includes all cancers except leukaemia.

^c 'All other' includes all cancers except leukaemia and bone.

^d 'All other' includes all cancers except those specified in the table.

used were the same as in Ref.[1] which are slightly modified from those presented in Ref.[2]. The risk factors, latent periods and plateau periods are presented in Table III (from Ref.[1]).

The reduction in life expectancy for the linear model, the dose-effective factor model with acute and chronic exposure, the quadratic model and the cube-root model are presented for one rem exposure with several selected ages and for the whole population in Table IV. The reduction in life expectancy is also presented for the whole population as a function of dose in Fig.2. Table V presents the average years lost per fatality for fixed latent periods and for the cube root model with 1 and 100 rem. Table VI presents the linear risk factors

TABLE IV. REDUCTION IN LIFE EXPECTANCY (in days)
FROM 1 rem EXPOSURE TO CAESIUM-137

Age	Linear [2]	Dose eff. fac. [1]	Quadratic [9]	Cube root [13]
In utero	9.73	1.95	0.020	9.73
25	3.12	0.62	0.009	0.50
50	0.74	0.15	0.002	0.07
Total population	1.62	0.32	0.004	0.46

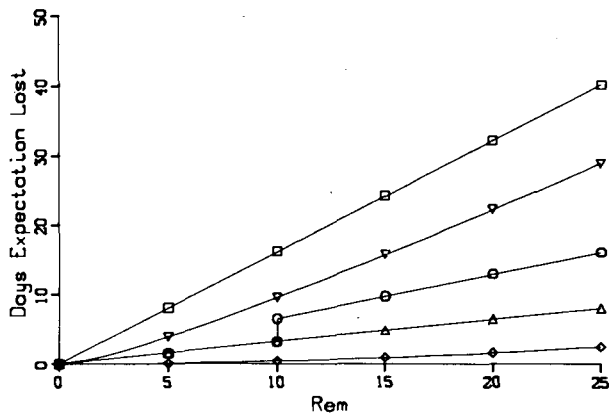


FIG.2. Reduction in life expectancy.
□ Linear
○ Dose effectiveness factor: acute
△ Dose effectiveness factor: chronic
◇ Quadratic
▽ Cube-root latent period

for computing the expected years lost for an individual as a function of the dose delivered (in rem) to the site at risk (e.g. for leukaemia, to the red marrow).

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TABLE V. AVERAGE YEARS LOST PER FATALITY (in years)
The expectation for an unexposed population is 38.8 years

Cancer type	Fixed latent period	Cube root [13]	
		1 rem	100 rem
Leukaemia	28.5	16.7	26.7
Lung	13.1	0 ^a	10.4
Stomach	13.1	0 ^a	10.4
G.I.	13.1	0 ^a	10.4
Pancreas	13.1	0 ^a	10.4
Breast	13.1	0 ^a	10.4
Bone	21.4	2.1	18.8
Other	19.3	0.7	15.7

^a No fatalities.

TABLE VI. LINEAR FACTORS FOR REDUCTION IN
 LIFE EXPECTANCY (in years) FROM ONE REM
 EXPOSURE TO THE SITE AT RISK

Cancer	Reduction
Leukaemia	1.04×10^{-3}
Lung	6.48×10^{-4}
Stomach	2.99×10^{-4}
G.I.	9.97×10^{-5}
Pancreas	9.97×10^{-5}
Breast	7.48×10^{-4}
Bone	2.13×10^{-4}
Other	7.06×10^{-4}

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DISCUSSION

P.G. GROER: In the first part of your paper you consider different models for dose-response, and in the second part you use these models in a competing risk framework to calculate the resulting reduction of life expectancy. But the models you used are not the result of a competing risk analysis.

H.T. DAVIS: The models used were for excess cancer deaths, and this does reflect the competing risk problem. The mortality tables published in HEW [15] were used to estimate, for each model, the risk-free model (the probability of L_i).

CANCERS PULMONAIRES INDUITS PAR DIFFERENTS EMETTEURS ALPHA INHALES

Evaluation de l'influence de divers paramètres et comparaison avec les données obtenues chez les mineurs d'uranium

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Abstract—Résumé

LUNG CANCERS INDUCED BY VARIOUS INHALED ALPHA EMITTERS: EVALUATION OF THE EFFECT OF VARIOUS PARAMETERS AND COMPARISON WITH DATA OBTAINED FROM URANIUM MINERS.

Experimental studies have been carried out since 1970 on the carcinogenic effect of inhaled alpha emitters. The effects of radon and of actinides have been compared on rats of a common origin. Agreement has been observed between the risk values obtained with radon and those found from epidemiological studies on uranium miners. The dose-effect relationship is not linear, and the incidence per unit dose increases as the total dose is reduced. It has also been found that, when the inhaled actinides are soluble, cancers occur in other organs, and estimates of the incidence of these as a function of dose have been made.

CANCERS PULMONAIRES INDUITS PAR DIFFERENTS EMETTEURS ALPHA INHALES: EVALUATION DE L'INFLUENCE DE DIVERS PARAMETRES ET COMPARAISON AVEC LES DONNEES OBTENUES CHEZ LES MINEURS D'URANIUM.

Depuis 1970 des études expérimentales ont été entreprises sur l'action cancérogène des émetteurs alpha inhalés. Sur des rats de même origine on a pu comparer l'action du radon et celle des actinides. On a observé une concordance entre les valeurs de risque obtenues avec du radon et celles tirées des enquêtes épidémiologiques sur les mineurs d'uranium. La relation dose-effet n'est pas linéaire, et l'incidence par unité de dose augmente quand la dose totale diminue. On a obtenu aussi, quand les actinides inhalés étaient solubles, des cancers d'autres organes pour lesquels on a fait des estimations sur l'incidence en fonction de la dose.

En 1970, Perraud [1], Chameaud et al. [2] montrent qu'il est possible, chez le rat, d'obtenir des cancers pulmonaires avec des inhalations de radon à des taux comparables à ceux inhalés par certains mineurs d'uranium. Depuis cette date, de nombreux travaux expérimentaux ont été entrepris dans plusieurs laboratoires du Commissariat à l'énergie atomique (CEA), en association avec la Communauté européenne (Euratom), pour étudier les cancers pulmonaires provoqués par l'inhalation d'émetteurs alpha.

L'objectif était de comparer aux résultats obtenus avec du radon ceux fournis par les actinides et d'étudier l'influence de divers paramètres physiques et biologiques. La comparaison avec les données humaines chez les mineurs d'uranium pouvait permettre, par analogie, de prévoir les risques liés à l'inhalation d'actinides.

Avec l'inhalation d'actinides solubles (^{241}Am , ^{244}Cm) on a observé des cancers de nombreux organes, et un programme complémentaire a été lancé pour comparer, après irradiations externes globales, la radiosensibilité des différents organes d'animaux de souches identiques, maintenus et analysés dans les mêmes conditions expérimentales.

1. EFFETS CANCEROGENES DES ACTINIDES INHALES CHEZ LE RAT

1.1. Cancers du poumon

Le tableau I résume les fréquences observées de cancers en fonction de la dose totale. Plus de 200 cancers ont été analysés après inhalation de ^{239}Pu et plus de 100 avec ^{241}Am . Chez le témoin, le taux d'apparition spontanée est de l'ordre de 1%.

Le tableau II exprime l'incidence par unité de dose (rad) en fonction de la dose totale.

Compte tenu des erreurs statistiques, les données du tableau I, à l'exception des deux dernières de chaque groupe, peuvent s'ajuster sur des fonctions linéaires. Par contre, les données du tableau II montrent à l'évidence que la loi qui relie la dose et la fréquence ne peut être une fonction linéaire.

L'incidence par unité de dose est donc d'autant plus élevée que la dose totale est plus faible. Pour des doses totales de l'ordre de la centaine de rad, cette incidence serait de 1000 par 10^6 et par rad, ce qui, avec un EBR de 20, donnerait pour les particules à faible TEL une incidence de 50 par 10^6 et par rad, tout à fait comparable à l'estimation du Rapport de l'UNSCEAR [3].

1.2. Cancers extrapulmonaires

Avec les actinides solubles, on observe des cancers du squelette et des autres organes. Fait surprenant, on n'a pas observé une augmentation significative de la fréquence des leucémies.

TABLEAU I. CANCERS DU POUMON
APRES INHALATION D'ACTINIDES

	Doses (rad)	Fréquences
^{239}Pu	200	10 : 52
	600	25 : 98
	2 000	76 : 139
	6 000	85 : 175
	20 000	22 : 81
^{241}Am	150	18 : 132
	600	38 : 103
	2 000	47 : 106
	6 000	9 : 33

TABLEAU II. ACTINIDES: INCIDENCES
DES CANCERS PULMONAIRES PAR 10^6
ET PAR RAD

	Dose (rad)	Incidences
^{239}Pu	200	1000
	600	420
	2 000	270
	6 000	80
	20 000	15
^{241}Am	150	900
	600	600
	2 000	220
	6 000	50

TABLEAU III. ACTINIDES SOLUBLES:
INCIDENCES DES CANCERS OSSEUX PAR
10⁶ ET PAR RAD

Doses (rad)	Rats	:	Cancers	Incidences
750	15	:	6	530
350	130	:	28	615
160	42	:	4	595
110	56	:	3	490
35	103	:	2	550

TABLEAU IV. CARCINOMES
BRONCHOGENIQUES
(500 observations)

	Bronchioles	Bronches
Actinides	230	4
Radon	248	18

1.2.1. Ostéosarcomes

Le tableau III résume les résultats. Il montre une incidence constante par unité de dose pour des doses totales comprises entre 35 et 750 rad. Cependant, le nombre de cancers observés à des doses inférieures à 350 est trop faible pour que cette conclusion soit indiscutable.

L'incidence par unité de dose est élevée: 500 par million et par rad si on la compare aux valeurs humaines, mais le squelette du rat est en croissance continue pendant la durée de la vie, ce qui augmente certainement le risque.

1.2.2. Autres cancers

Après le squelette, deux organes, le foie et le rein, reçoivent des doses plus élevées que la moyenne. La fréquence des cancers du foie n'est pas augmentée par rapport aux témoins. Les cancers du rein ne sont augmentés que chez le rat Sprague-Dawley et non chez le rat Wistar. Le rein du Sprague-Dawley présente une malformation congénitale et est anormalement radiosensible.

TABLEAU V. EFFETS SUIVANT LA DOSE:
DEBITS DE DOSES INFERIEURS A
1000 WLM/SEMAINE

Doses (WLM)	Survies (d)	Cancers (fréquences)	Latences (d)
175	660	11 : 294	742
900	670	4 : 20	770
1500	610	5 : 20	690
3000	670	17 : 40	710
4000	600	17 : 50	650
6000	620	42 : 80	650
8000	560	73 : 200	610

TABLEAU VI. EFFETS SUIVANT LA DOSE:
DEBITS DE DOSES SUPERIEURS A
1000 WLM/SEMAINE

Doses (WLM)	Survies (d)	Cancers (fréquences)	Latences (d)
2 200	600	7 : 25	690
3 700	530	8 : 25	570
5 400	450	6 : 25	580
7 000 (1)	400	8 : 26	500
7 000 (2)	430	11 : 37	530
20 000	180	3 : 50	210

Le nombre des cancers de tous les autres organes, traités en bloc, est trop élevé, même à des doses totales de quelques rad seulement.

Il n'est pas possible d'étudier la radiosensibilité de chacun des organes et l'estimation de risque pour une contamination alpha généralisée et à concentration homogène ne peut être très précise.

Une valeur de 20 000 par million et par rad est raisonnable.

TABLEAU VII. CANCERS DU POUMON:
INCIDENCES PAR 10^6 ET PAR WLM –
FAIBLES DEBITS DE DOSES

WLM	Incidences
175	210
900	220
1500	170
3000	140
4000	85
6000	90
8000	45

2. EFFETS CANCEROGENES DU RADON INHALE CHEZ LE RAT

2.1. Localisation des cancers pulmonaires

Le tableau IV compare les localisations périphériques ou centrales des cancers de type bronchogénique observés après inhalation d'actinides ou de radon. Avec le radon, pour lequel la dose locale maximale est délivrée aux voies respiratoires supérieures, on n'observe qu'une faible augmentation de la fréquence relative de cette localisation des cancers.

La localisation semble dépendre beaucoup plus de l'espèce animale que de la surdose locale. Félins et rongeurs font des cancers en majorité périphériques, alors que les primates ont des cancers centraux [4–6]. Par contre, on observe de bonnes concordances inter-espèces dans les relations dose-effets.

2.2. Relations dose-effets pour des cancers pulmonaires

Les tableaux V et VI résument les résultats pour deux débits de dose différents. Ils montrent qu'aux forts débits de dose, la fréquence des cancers est plus faible pour une même dose totale.

Les tableaux VII et VIII résument, aux deux débits de dose, les incidences par WLM des cancers pulmonaires en fonction de la dose totale. Comme pour les actinides, cette incidence croît quand la dose totale diminue.

Le rapport de l'UNSCEAR [7] souligne les différences entre les données américaines et celles obtenues en Tchécoslovaquie, qui indiquent une incidence plus élevée dans ce pays. Plusieurs hypothèses expliquant ces différences ont été avancées (âge, co-facteurs, etc.). Il est possible que, comme chez le rat, ces différences ne tiennent qu'aux différences de dose totale.

TABLEAU VIII. CANCERS DU POUMON:
INCIDENCES PAR 10^6 ET PAR WLM –
FORTS DEBITS DE DOSES

WLM	Incidences
2 200	130
3 700	90
5 400	45
7 000 (1)	45
7 000 (2)	40
20 000	3

TABLEAU IX. EFFETS SUIVANT L'AGE
POUR 4 GROUPES DE 25 RATS A
6000 WLM

Ages à T_0 (d)	Survies moyennes (d)	Nombre de cancers	Latences moyennes (d)
150	575	14	640
280	420	11	510
400	350	6	450
520	250	4	305

2.3. Effet de l'âge au début de la contamination

Le tableau IX résume une expérience destinée à étudier les effets de l'âge. Il montre deux résultats intéressants. Lorsque l'on attend le décès de tous les animaux, on observe que la fréquence totale est d'autant plus faible que l'âge au début des inhalations est plus avancé, mais la latence moyenne est d'autant plus courte que l'âge est plus avancé. Ce deuxième point implique que si l'on étudie une population de rats d'âges différents, contaminés en même temps, pendant un intervalle de temps court par rapport à la durée de vie moyenne de l'espèce, on observera que l'incidence des cancers est d'autant plus élevée

TABLEAU X. CANCERS DU POUMON CHEZ
LES MINEURS D'URANIUM
(RAPPORT UNSCEAR 1977)

Age au début du travail	Incidence par 10 ⁶ et par WLM
Moins de 30 ans	140
30 à 39 ans	230
40 ans et plus	370

que l'âge au début de l'inhalation était plus avancé. Cet effet diminue quand la durée de l'étude augmente et s'inverse quand tous les animaux sont morts.

Un résultat comparable a été observé dans une population de mineurs tchécoslovaques suivie pendant une vingtaine d'années seulement [8] (tableau X).

2.4. Autres cancers

On n'a observé chez les animaux ayant inhalé du radon qu'un excès de la fréquence de deux autres cancers. L'augmentation de la fréquence des cancers des voies urinaires est due à la grande radiosensibilité de ce système chez le rat Sprague-Dawley.

Par contre, l'augmentation de la fréquence des épithéliomas de la lèvre supérieure est indiscutable (5 cas sur 2000 animaux contre 0 pour 4000 rats dans les expériences où les animaux n'ont pas inhalé de radon).

Une augmentation de ce type de cancer a été aussi signalée chez l'homme [9].

3. PROBLEMES POSES PAR L'ESTIMATION DES RISQUES CHEZ LES RONGEURS SOUMIS A DES INHALATIONS D'EMETTEURS ALPHA

Par rapport aux estimations faites chez l'homme, les données obtenues chez les rongeurs montrent une bonne concordance en ce qui concerne les poumons et une grosse différence pour les autres cancers.

Dans les deux cas, le problème expérimental de la validité des estimations doit se poser en considérant la relation entre la dose totale et l'incidence par unité de dose. L'approche basée sur la relation linéaire entre la dose et la fréquence aboutit à une sous-estimation du risque. En effet, si nous considérons la relation entre la dose et l'incidence par unité de dose, nous disposons de

points expérimentaux qui montrent que cette incidence décroît quand la dose croît. D'un autre côté nous savons que, quand la dose est nulle, l'incidence l'est aussi. La courbe part donc de zéro, monte vers un maximum puis redescend à zéro aux doses les plus élevées.

Il y a donc deux problèmes: d'une part, définir les valeurs en dose et en incidence du maximum et, d'autre part, préciser la partie croissante de la courbe (problème du seuil).

Mais, de toute façon, le risque est sous-estimé si, au lieu de l'apprécier sur *la* valeur du maximum, on utilise une fonction linéaire qui fait la moyenne de plusieurs doses.

3.1. Poumons

Expérimentalement, étant donné les fréquences de plus en plus faibles vers lesquelles on descend quand la dose diminue, la limite que nous pouvons techniquement atteindre est de faire inhaler à un groupe de 500 animaux une dose moyenne entre 50 et 100 WLM. On peut en attendre entre 5 et 10 cancers du poumon.

Les données humaines dont on dispose [10] ne laissent que peu d'espoir d'avoir une incidence par unité de dose plus faible à ces niveaux. Descendre en dessous de ces valeurs nécessiterait des lots d'animaux trop importants.

Expérimentalement, on peut aussi ajouter des co-facteurs pour sensibiliser les animaux. Dans ce cas, les études préliminaires sont longues et la possibilité de progrès est plus liée à un hasard heureux qu'à une analyse rigoureuse des phénomènes.

La seule solution qui, à notre sens, peut permettre de résoudre le problème est de comprendre, puis de quantifier, les divers phénomènes qui interviennent dans l'apparition des cancers.

3.2. Autres organes

Les premiers résultats que nous avons obtenus, avec des irradiations globales, sont trop partiels pour que des valeurs précises puissent en être tirées. On observe déjà, cependant, qu'il y a une bonne concordance entre les données pulmonaires et celles obtenues avec les émetteurs alpha.

De plus, la concordance existe aussi pour les autres cancers, pris en totalité.

Expérimentalement, le problème est le même que pour les poumons. Néanmoins, comme le nombre d'organes est plus grand, la fréquence d'apparition pour une même dose est plus élevée. La sensibilité globale est donc meilleure et on peut espérer, avec des lots raisonnables d'animaux, descendre à des doses de quelques dizaines de rem.

CONCLUSION

Les études expérimentales ne pourront pas, par l'observation directe, donner des valeurs de risque pour des doses inférieures au rem. Cependant, les concordances et les discordances que l'on observe entre les données expérimentales et les estimations de risque chez l'homme montrent que les expériences peuvent être utilisées pour critiquer certaines hypothèses biologiques sur lesquelles sont basés les calculs, à condition que les différences qui peuvent exister entre les mammifères soient scientifiquement établies et non postulées.

Néanmoins, il nous semble beaucoup plus probable que la solution du problème des effets des faibles doses sera trouvée, si elle doit l'être, à partir d'expériences sur les mécanismes fondamentaux qui interviennent dans la cancérogenèse radio-induite.

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DISCUSSION

W.A. MÜLLER: I can confirm your results from our experiments with short-lived bone seekers. In particular, we found that the latency period decreases with increasing age, and low doses are more effective per rad applied. Do you think there could be a general rule here?

J. LAFUMA: I would not like to say.

J.R. MAISIN: If I understand you correctly, you said that if a species or a strain is sensitive to a particular type of cancer, for example bronchial cancer, then, no matter where a radionuclide is located in the lung (alveoli or bronchi), the animals are assumed always to contract that type of cancer. Is this in fact correct?

J. LAFUMA: Yes, in more than 95% of cases.

D. BENINSON: From your presentation it would appear that the relevant dose for assessing risks from lung irradiation is the average dose to the entire organ. Could you please comment?

J. LAFUMA: If one compares the results derived from inhalations of ^{239}Pu oxide in rodents, dogs and primates, the values obtained are very close if the doses are referred to the mass of the entire lung.

A.B. DORY: Could you speculate on the effect that a reduction in dose would have on the latency period in humans compared with the effect observed in animals?

J. LAFUMA: We are currently investigating this question on baboons, which are primates like man and which have a life-span of around 30 years. Only by studying this effect in a primate will we be able to predict what will happen in man.

ROUND TABLE DISCUSSION

SUMMING UP AND FUTURE PROSPECTS

Round Table Discussion

Members of the panel: A.S. McLEAN (*Chairman*)
H. ALTMANN
K.H. CHADWICK
M. COLMAN
S. KOBAYASHI (*Scientific Secretary*)
T. SADO
L.B. SZTANYIK
R.L. ULLRICH
B.C. VON BORSTEL
J.L. WEEKS

A.S. McLEAN (*Chairman*): First, the Chairman (or rather one of the two Chairmen) of each of the sessions held during the week will in turn summarize what they regard as the highlights of their respective sessions, and in two cases they will also be reporting on the evening sessions which they also chaired. After that we shall have a general discussion in which all are invited to participate. I will call first on Mr. Ullrich, who was Chairman of the session on Animal Experimentation, Part 1, to present his summary.

R.L. ULLRICH: The main aim of experimental studies on the late effects of radiation is to provide information that may be useful for the evaluation of risk to human beings from radiation exposure. This may be accomplished through studies on the influence of dose, dose rate and radiation quality on such effects, as well as through studies on the biological bases for these influences. With such an experimental approach as opposed to a more empirical approach it is likely that general principles can be derived which are applicable for estimating human risk. The papers in the first session on animal experimentation in the main consisted of reports on studies which, though in various stages of development or completion, have taken this experimental approach. Life-span studies as well as studies on specific model systems or effects on specific organ systems were presented.

First, I will discuss the life-span studies. The paper by Spalding was a report on early findings of a study designed to examine the importance of genetic factors on the influence of dose, dose rate and age in radiation carcinogenesis. Sado and co-workers compared the natural and radiation-induced tumour incidence in two genetically different strains of mice in order to understand the mechanisms of radiation tumorigenesis. Information on dose and dose rate effects

in dogs was provided by Fritz and his associates. It is hoped that, as more information from their investigations becomes available, comparison of these studies with studies in mice will be made. By ascertaining the similarities and understanding the basis for differences between strains of animals of the same species and between different species, a better understanding can be obtained of the processes involved in radiation carcinogenesis, and more reliable estimates can be made of the risk to humans.

Grahn and his co-workers presented details of an analytical approach to data which appears to provide a means for comparative analysis of dose, dose rate and risk factors among animals of different sexes and genetic characteristics, i.e. different strains of mice and even different species. It was proposed that certain similarities found among strains and species by using this analytical approach might provide some basis for extrapolation of risk.

It is hoped that the life-span studies performed by Ullrich and Storer will also be useful for the understanding of the influence of dose, dose rate and radiation quality in carcinogenesis.

Studies were also reported that examined tumorigenic effects in model systems and late biological effects in particular organ systems. The experiments of Coggle and Peel, using the lung as a model system, were interesting. These studies suggested differences in tumour induction after uniform and non-uniform radiation exposure of the lung, which would imply that the use of average tumour tissue dose in situations of non-uniform irradiation may underestimate the tumour risk. However, it was emphasized by Coggle that these studies are not yet complete, and further work is necessary before the applicability of these data to problems such as the hot-particle problem can be confirmed.

Two studies on the influence of radiation on mammary tumour induction were presented. Broerse and co-workers have produced preliminary information on an experiment in progress to study the nature of the dose response for X-rays and neutrons, and the interaction of oestrogen and neutron irradiation, and to determine the RBE of neutrons in three strains of rats with different susceptibilities to tumour induction. The use of different strains with different levels of susceptibility to tumour induction is essential if we are to obtain reliable general principles for estimating the risk for human beings. Vogel presented data on mammary tumorigenesis in Sprague-Dawley rats. In this paper the results of effects of high LET radiation other than neutron were presented as well as new data suggesting that doses of neutrons divided into two equal fractions separated by 24 hours were as effective as similar single doses. In addition, data on neutron RBE for the induction of mammary tumours in Sprague-Dawley rats obtained by various investigators were reviewed. These data, as we all know, indicate that RBE increases with decreasing dose as a result of the different shapes of the neutron and X-ray dose response curves.

In addition to the studies on tumour induction, work on late biological effects in organ systems was also reported. The studies of Sado and co-workers and the elegant stereological studies of Kalisnik and co-workers on endocrine organs and the thymus provide very useful information on non-neoplastic late biological effects. These findings may also prove useful in understanding the role of the immune system and the endocrine system in carcinogenesis.

Finally, lest we forget that neoplasia is not the only target of radiation, particularly in clinical settings, the study of Calvo and his co-workers presents evidence of radiation-induced fibrosis in bone marrow.

In the first evening discussion three problems were briefly discussed:

(1) the competing risk problem in the analysis of animal data; (2) neutron RBE factors; and (3) mammary tumour induction and, more generally, the influence of endocrine and other co-factors in tumour induction. Cumulative incidence may not give a true measure of risk because of different rates and causes of mortality between groups. More attention should be paid to these differences and appropriate adjustments made for competing risks using available methodology when reporting and interpreting data on radiation carcinogenesis. Methods discussed included direct age adjustment, measures of displacement of life table parameters, and the Kaplan-Meier correction procedure. These procedures allow better comparison of experimental factors, e.g. dose, dose rate and genetic variation.

The low dose neutron RBE question was discussed only briefly, as all the available information on neutron effects was not presented. The present position of the standard-setting commissions was carefully noted, and it was concluded that present standards appeared to be generally adequate but there was a need for thoughtful review which could not be accomplished in the present forum.

Because of shortage of time the discussion on co-factors in tumour induction was rather brief. It was generally felt that insufficient information is available at present, and that more investigations of the influence of the endocrine system and other co-factors in tumour induction and their modification of dose response curves are needed.

A.S. McLEAN (*Chairman*): May I now invite Mr. Sado to give his impressions of the second session on Animal Experimentation.

T. SADO: The second session on Animal Experimentation included nine papers dealing with three different aspects of the late effects of radiation, namely tumour induction, lesions of the reproductive organs and lesions of the central nervous system. It is felt that Mr. Vogel's paper on the induction of mammary tumours by high LET radiation would fit better into the session just reviewed by Mr. Ullrich.

The paper by Van Zwieten and co-workers emphasized the high incidence of tumours in neutron-irradiated, bone-marrow treated monkeys. As most control animals have not yet died, one cannot decide whether this finding is related to a

shortening of the latency period or to an actual increase in tumour incidence. Nevertheless, it seems noteworthy that no leukaemia was found in this group and that one glioma was observed. As such rare central nervous system tumours were also observed in the group of monkeys examined by Mr. Caveness, one might wonder whether monkeys are specifically sensitive to induction of such tumours following severe radiation damage to this organ. It might be worthwhile, therefore, to conduct an experiment with a similar irradiation regime in rodents to see whether or not the development of tumours of the central nervous system is unique in monkeys.

We are also looking forward to the results of further studies on the fate of X-irradiated, bone-marrow treated monkeys, as the bone-marrow therapy combined with whole-body X- or gamma-irradiation appears to have become a more frequent clinical practice in recent years for treating various haematological disorders.

The paper by Phemister and co-workers was an attempt to evaluate the sensitivity of beagle dogs to the carcinogenic effects of low-level radiation delivered during pre- and post-natal periods. Results showed a high incidence of tumours of various types. There is no doubt that this corresponds to a shortening of latency of tumour development, but it is too early to know what kind of malignancies were really induced. Further studies on these animals should provide the answer to this important question.

The paper by Erickson and Reynolds emphasized the importance of studying female reproductive performance in animal models that can be closely related to the human situation. The authors specifically drew attention to the low sensitivity of the bovine ovary to acute radiation exposure, and concluded that the oogonia of long-lived mammals appeared to be more resistant than that of short-lived mammals. Thus, it seems that the situation which applies to long-lived mammals is just the opposite to that existing in rodents. The authors consider that this is most likely because of the greater time allotted to the long-lived mammals to compensate for losses of oogonia due to radiation exposure. We must keep this in mind when we are attempting to extrapolate animal data to man.

I would add, however, that this does not necessarily mean that basic knowledge obtained from short-lived mammals is less significant.

In the paper by Caveness and Carsten the application of a simulated human radiation therapy regime to *Macaca* monkeys of different ages was described. We consider that the results obtained from this extensive and careful pathological study provide an interesting base line for the study of the late effects of brain irradiation in man.

The papers by Dufour and Keyeux both dealt with cerebral circulation after irradiation. Although the former used thermal conductivity and worked with rabbits and the latter used radioactive tracers in rats, they both reached the conclusion that the primary modification is an increase in cerebral blood flow.

According to these authors this circulatory change preceded any degenerative or occlusive alteration of the cerebral blood vessels.

If the techniques employed in these studies were applied to the monkeys studied by Mr. Caveness, the results obtained would give us a much better insight into the radiation injuries of the brain that lead to the manifestation of lesions. Both Mr. Duplan, co-chairman of this session, and I share the view that inter-laboratory collaboration in research of this nature should be encouraged.

A.S. McLEAN (*Chairman*): Mr. Chadwick was Chairman of the session on Indicators for Late Effects, and I wonder what he thought about that.

K.H. CHADWICK: Mr. Covelli, the co-chairman of this session, and I have chosen to emphasize the subjects that we felt personally to be most relevant for the indication of late effects. This does not detract from the importance of the other work presented in this session which is not explicitly mentioned here.

The measurement of DNA repair was covered in two papers in this session, by Riklis and Kol and Altmann and Tuschl respectively. In both papers the authors investigated the ability of lymphocytes of chronically exposed people to repair an additional DNA insult. The results appear to be contradictory, although this might be due to methodological differences. It is interesting to note that Altmann and Tuschl reported an increased level of repair in people working in high-background areas. We would like to see more work of this type.

The papers of Kirsch-Volders and co-workers and Brandom and co-workers dealt with the effects of low-level exposure on cytological indications. Kirsch-Volders reported 'partial band loss' in the chromosomes of the blood of babies exposed in X-ray pelvimetry examinations. Brandom reported on the induction of aberrations in uranium miners and plutonium workers. One important conclusion from this work was that dicentrics and rings alone did not provide a satisfactory indication, and that therefore all aberrations should be scored. We do not accept the dismissal of these results by Dolphin on dosimetry grounds. We do appreciate the problems posed by Dolphin but find the results on the plutonium workers particularly impressive. Some association may be made between these results and a comment made later in the meeting by Littlefield who suggested that the low-level RBE for ^{241}Am alpha particles might be as high as 30–40.

We appreciated the pragmatic approach of Păun and co-workers who have a system for withdrawing uranium miners from high-exposure areas in the event of cytological indications of metaplasia and a high radioactivity level of the sputum – a fine example of preventive medicine.

Fehér presented an elegant measurement technique that enabled him to demonstrate residual damage in the haematopoietic system of animals after relatively low doses of chronic and acute radiation.

Erflé emphasized the role of viral aetiology in the radiation carcinogenesis in animals. The cell-free transmission of the virus needs further investigation.

This was an interesting session and it is clear that there is still a great deal of work to be done in this field. We should prefer efforts to be concentrated on DNA repair and cytological investigations.

A.S. McLEAN (*Chairman*): Now, please may we hear from Ms. Altmann.

H. ALTMANN: Together with Mr. Burns I chaired Session VIII dealing with the Theoretical and Experimental Study of Dose-response Relationships for Late Effects.

The session started with an analysis of the dose-effect relationship for cancer induction by Barendsen. Malignant transformation of cells as well as cell reproductive death, which can prevent the development of these transformed cells into malignant tumours, involve components which increase in part as a linear function and in part as a quadratic function of the dose. Some models of the dose-response relationship presented in this session showed a good correlation between experimental data and theoretically derived equations, whereas others are in contradiction. Therefore different values of the parameters must be used for different types of tumours. The spontaneous mutation rate and the rate doubling concept for man are troublesome to handle and it is difficult to make general statements, because the 'resistance' of man to different parameters depends on several factors. One of the most important factors seems to be the DNA repair process. These mechanisms seem to work mainly error-free, because a detectable threshold dose exists for mutation and the spontaneous mutation rate is a 'background noise' level from which the induced non-threshold effect will start (R.C. von Borstel). A quantitative experimental study of low-level radiation carcinogenesis involves many questions such as the naturally occurring tumour spectrum, different latency periods and interference with other diseases. The data of Mewissen and co-workers are consistent with the existence of an inter-competitive process of tumour mutation and development that is triggered or accelerated by radiation, and the accelerations of different tumours and also of various diseases are different. The response is predominantly non-linear. The tumour-host/animal index and the tumour/host index tend to decrease at lower doses, but this tendency is reversed at higher doses, especially after neutron irradiation.

From chromosome aberration studies on lymphocytes from population groups living in an environment with variously high levels of natural radioactivity it can be concluded that the initial part of the dose-effect curve for chromosome aberrations has no threshold at the lowest dose but rises to about 300 mrad/a ($(\alpha + \beta)$ dose) and passes into a convex upward form with a plateau until it meets the linear curve of higher doses (J. Pohl-Rühling). The shape of this curve could be caused by an inducible error-free repair pathway. Chadwick and Leenhouts presented the interesting theory that radiation-induced DNA double-strand breaks are the most critical lesion which may lead to either cell death, chromosomal aberrations or somatic mutations. The difference between two population groups

in terms of cancer risk at low doses of radiation is dependent on whether people carry the malignant genotype that is normally suppressed, or do not carry it at all.

The recessive nature of the malignant genotype seems to be a general feature of mammalian eukaryotic cells. Tumour induction events at the cellular level in vivo were studied by Silini and co-workers. A certain qualitative relationship was found between the probability of in vivo transformation to potentially neoplastic cells and the radiation dose between 0 and 800 rads. Groer demonstrated that there is insufficient information in the observed survival functions to estimate the time distribution for one particular response, if the assumption of independence is dropped. For so-called incomplete data the shape of the dose-response curve is therefore indeterminate in some situations. Since the Kaplan-Meier estimates for incidence of cancer are different from traditional fractional estimates, the shape of the corresponding dose-response curve (or band) will be changed.

Sacher and co-workers reported on the relation of life-span shortening to the daily dose (12–125 R/day) applied to 15 species of mammals throughout adult life. The data can be accounted for by a mathematical model that postulates that cellular radiation injury is due to lethal chromosome rearrangements. It seems that there is little interspecies variance in the efficacy of repair of DNA damage produced by ionizing radiation. Two species which differ in life-span by a factor of 2 have a 2.5 fold difference in repair of UV lesions in DNA, but do not differ in the rate or amount of DNA repair after damage produced by ionizing radiation. The relationship between life-span and radiation variables appears to be applicable to tumour incidence as well. Cowper presented the paper by Myers and co-workers on DNA repair and the assessment of the biological hazards of ionizing radiation. The carcinogenic hazard of ionizing radiation seems to be related to a very small residue of the initial DNA damage which remains after excision repair. Human diseases such as ataxia telangiectasia are good examples of the close correlation between defective DNA repair and the occurrence of late effects. Because many environmental contaminants and also drugs can influence DNA repair processes, it is reasonable to assess the hazards of low-level radiation by linear extrapolation from measured effects at higher radiation level, to be sure that extrapolation is on the safety side of the calculations.

A.S. McLEAN (*Chairman*): Mr. von Borstel will now present his summary of Session IX, Combined Effects.

R.C. VON BORSTEL: The combined effects of radiation and other insults on cells and tissues require a comprehensive theory before any predictions become possible. Therefore it is pleasing to have had presented to us an array of experiments on combined effects which, when taken together, illustrate why more than one explanation for additivity, synergism and antagonism is needed.

The appearance of tumours depends on advancement of a specific mutation through several stages. Any of these stages is surrounded by a formidable array of biological defences which a tumour cell must overcome to become a cancer.

These stages represent, for example, tumour initiation, tumour promotion, clone establishment and tumour growth. Molecular, cellular, tissue, organ and organismal defences are erected at each level.

Combined effects of radiation and other agents can operate at any of these levels. Streffer demonstrated synergisms that resulted in hypoploidy and production of micronuclei. Michel spoke of synergism using developmental anomalies as the criterion. Leenhouts mentioned mutations and chromosomal aberrations. Morin, Chameaud, Lurie, Philip and Maisin told us about synergistic and antagonistic combined effects concerned with establishment of tumours.

So where does the action take place? Stewart favours a gradual deterioration of immunological defence systems. This notion deals with relaxation of barriers at the stage of clone establishment. Lurie spoke of increased vascularization from radiation and its independence from a similar phenomenon caused by application of DMBA; he is carrying out research at the level of breakdown of barriers against clone establishment and tumour growth. Morin and Chameaud and their collaborators are working at the level of breakdown at the tumour promotion level and clone establishment level, and possibly with the hypothesized release of endonucleases from lung cell lysosomes — they did not mention these; these are my own interpretations. Leenhouts prefers to think of synergism at the level of interaction of DNA structures, but research on combined effects by Leenhouts and Chadwick, as well as that by Streffer and Michel and their collaborators, could be looked at from the point of view of repair pathways.

A theory for prediction of interactions of two or more mutagens based on DNA repair is being developed. It is based on the following considerations which derive principally from investigations on bacteria and yeast: (1) there are a number of biochemical pathways which a cell can use to repair lesions in its DNA; (2) these pathways vary in complexity from one step to more than seven, and some of these pathways have branches. Also, some of the steps are used in common by some, if not all, of the pathways; (3) lesions in DNA are channelled into one of the pathways; (4) in yeast, any one of four different pathways can repair lesions induced by ultra-violet radiation, and two of these same pathways can repair damage induced by X-radiation; (5) some chemical mutagens induce lesions in DNA which are repaired by the radiation-repair pathways; (6) some chemical mutagens induce lesions which are repaired by entirely different pathways; (7) in yeast, five repair pathways are known for certain and at least two more are believed to exist; (8) two of these pathways are mutagenic and, while repairing the DNA, change the nucleotide sequence; (9) the other pathways are believed to be essentially non-mutagenic.

This information can be used to predict the combined mutagenic effects of any two agents which react with DNA or the repair pathway enzymes:

- (a) Any two mutagens will show additivity if the induced lesions are repaired by the same or different mutagenic repair pathways;

- (b) A synergistic effect is observed from two agents if one of them induces a mutagenic repair pathway or an activating enzyme system;
- (c) A synergistic effect is observed if one of the agents blocks a non-mutagenic repair system and the lesions are thereby channelled into a mutagenic repair pathway;
- (d) An antagonistic effect is observed if one of the two agents blocks a mutagenic repair pathway and the lesions are thereby channelled into a non-mutagenic repair pathway.

Maisin and Philip discussed the action of radioprotective agents. Certainly, a proportion of the protection afforded by radioprotective agents is based on action at the cellular level. Experiments have been carried out which show that mercaptoethanol exerts its full protective effects only at concentrations where cell division is blocked. Presumably, if an agent inhibits cell division during the time of irradiation, the cell has more time to repair damage by way of non-mutagenic repair pathways. This would satisfactorily explain protection against cancer. It is not known how far these notions can be carried to explain protection against such late effects as glomerulosclerosis.

In any case, I think it is clear that we cannot use any one area of breakdown of defences of the organism to explain interactive effects. Nevertheless, the hypotheses are now eminently testable and testing them will lead to predictability for interactive effects.

A.S. McLEAN (*Chairman*): I now call on Mr. Sztanyik to give us his impressions of Session I, which dealt with populations exposed to atomic explosions.

L.B. SZTANYIK: As you will recall, seven papers were presented in this session of which six dealt with the late consequences of A-bombing in Hiroshima and Nagasaki, and the remaining one with the delayed manifestations of accidental exposure of a Marshallese population to the radioactive fall-out from the Bikini test.

The six papers on A-bombing covered such questions as: (a) the occurrence, induction-rate and latency of leukaemia and other malignancies among A-bomb survivors; and (b) the mortality of A-bomb victims, of children exposed in utero, and of the offspring of exposed parents.

A statistically significant increase in the incidence of leukaemia, breast cancer, lung cancer, thyroid cancer and stomach cancer has been firmly established and there is a near-significant increase in the incidence of cancer in the salivary glands, oesophagus, urinary tract and lymphoid tissue. Accordingly, the most important tissues at risk in the case of whole-body irradiation with relatively small and moderately large doses of high-intensity radiation are the bone marrow and the other organs and tissues mentioned.

The data presented on leukaemia induction were obtained in the investigation of the Life Span Study cohort, established in 1950, or by examination of the

general population in both cities. Each of these approaches has its own advantages and disadvantages. However, if the results obtained by these methods are consistent, the validity of the conclusions will be considerably strengthened.

Another important subject of discussion was the relative biological effectiveness of neutrons in induction of leukaemia. As is known, two models have been developed for describing the dose-effect relationship. One of these assumes that the incidence of leukaemia increases linearly with both neutron and gamma-ray doses while in the other it increases with neutron dose and as the square of the gamma doses. Dose-response data can be fitted by either of the models. From the first model, however, a general RBE value of neutrons can be deduced which is independent of dose, whereas RBE values dependent on dose are produced by the second model.

Mortality studies on A-bomb survivors have not provided any evidence of radiation-induced excess mortality from diseases other than neoplasms, such as tuberculosis, cerebrovascular, cardiovascular and some other diseases. The validity of the observation or opinion that reduced mortality can be detected among A-bomb survivors if compared with the Japanese average, has been disputed by some of the speakers.

As regards the latency of radiation-induced malignant diseases, investigations have shown that radiogenic leukaemias appear earlier than spontaneous leukaemias among members of the age-matched cohort. On the other hand, radiogenic cancers of the lung and female breast have shown no deviation in the time distribution from the spontaneous cancers. It has been suggested that fundamentally different mechanisms of carcinogenesis are manifested in these two patterns of behaviour.

A detailed analysis has revealed that the excess mortality of children exposed in utero is attributable, at least in part, to the mechanical injury of the mother. There is so far no dose-associated increase in mortality from leukaemia and other cancers. No significant effect of parental exposure has been demonstrated in the survival of the offspring either.

Finally, data on the delayed manifestations of accidental exposure of the Marshallese people to radioactive fall-out have indicated that, in addition to thyroid nodularity, which is a recognized effect of exposure of the thyroid to radioactive iodine or external radiation, biochemical evidence of thyroid dysfunction can appear as long as 25 years after irradiation of the thyroid with doses of about 350 rads, a fact which certainly deserves attention.

A.S. McLEAN (*Chairman*): Mr. Colman will now review the highlights of the two sessions on Late Effects due to Medical Exposure.

M. COLMAN: Against the background of laboratory and theoretical considerations and observations on exposed persons we have to ask how and in what amounts ionizing radiation can be justifiably used for medical purposes. Much information and guidance for the future has been gained as a result of errors in the past, emphasizing the need to record accurately what we do with

X-rays and other radiations, and to observe continuously the short- and long-term effects.

The sessions on Late Effects due to Medical Exposure included papers on several different topics. Rada, Mayfield and co-workers, Kuhn and co-workers and Correns and co-workers reported on the late effects of therapeutic irradiation for malignant conditions. Shimaoka and Sokal reported an unusual incidence of chronic myelogenous leukaemia in young patients treated for benign or malignant conditions or diagnostic purposes. Neumeister and Wässer reported the incidence of tumours in children after diagnostic X-ray examination of pregnant women. Boice and Stone reported on the incidence of breast cancer following multiple fluoroscopic exposures. Bailer (mammography) and van Kaick and co-workers (thorotrast) also reported malignancy following diagnostic techniques. Smith and Doll, Colman and co-workers, Neumeister and Correns, and Schmitz-Feuerhake and co-workers reported tumour incidence following therapeutic irradiation of benign conditions.

In the course of Smith and Doll's presentation and the discussion which followed the point was made that the treatment of ankylosing spondylitis by radiation may well still be justified in spite of the risk of malignancy because of the benefits to patients suffering from an often severely debilitating disease. From the views expressed in the other discussions it would seem that the treatment of benign conditions by therapeutic doses of radiation is not justified in the light of present knowledge of tumour induction and in view of the other methods of treatment available for those conditions.

Complications in the treatment of malignant tumours with radiation were reported by Correns and co-workers in the case of eye tumours, Rada in the case of cervix carcinoma and Mayfield and co-workers in the case of neuroblastoma. It was apparent that therapeutic techniques need to be improved in order to reduce complications, but that the use of radiation in these clinical situations is quite clearly justified and that the benefits outweigh the risks. Modern techniques, including careful treatment planning of dose distributions and the use of megavoltage equipment, are helping to reduce complications.

Bailer examined the use of mammography as a diagnostic screening procedure, and his studies provided valuable information indicating that the increased risks of the procedure as applied in the 1960s did not justify the benefits except in women over 50 years of age with a history of breast tumours or a family history of breast cancer. In younger women without these factors the risks were excessive. The use of modern techniques, newer equipment, faster screen-film combinations and magnification methods may reduce such risks. We feel that the whole situation ought to be carefully appraised in the light of this report. Boice and Stone reported a high risk of breast cancer in women exposed to fluoroscopic chest X-ray during air collapse therapy of the lung. Although this method of treatment is no longer in use, their finding of increased risk associated with menarche and

pregnancy is important, and has other implications for the safe use of X-rays in medicine.

The results of the long-term follow-up by van Kaick and co-workers of patients who had received thorotrast showed a significantly higher incidence of liver tumours and myeloproliferative diseases than in a control population. Thorotrast is no longer in use and the study outlined the value of carefully maintained records in retrospective studies of this type.

Colman and co-workers reported on the incidence of thyroid and other tumours following therapeutic irradiation to the head or neck area for benign conditions. They demonstrated an excess incidence of thyroid, salivary gland and brain tumours, but lacked an adequate control sample. Schmitz-Feuerhake and co-workers analysed the data of several authors relating to radiation-induced thyroid tumours, and demonstrated a well-marked dose-response relationship extending up to about 1500 rads. The shape of the curve beyond this level could not be determined because of lack of information. The available information was compatible with either a linear response function or a quadratic function, but there did not appear to be a threshold level. The findings of Neumeister and Wässer re-emphasized the importance of avoiding X-ray examinations during pregnancy.

These two sessions contributed some important new data which should be useful in establishing a balance between the risks of damage due to ionizing radiation and the benefits of its use.

A.S. McLEAN (*Chairman*): I now call on Mr. Weeks to report on the sessions dealing with Occupational Exposure.

J.L. WEEKS: Occupational exposure to radiation was considered in two sessions, a panel discussion and an informal evening discussion. Papers dealing with specific aspects of acute and chronic radiation injury were presented, together with a group of papers dealing in the main with the epidemiological aspects of such lesions.

The identification of specific lesions occurring in radiation workers and attributable to their occupation is important not only in relation to the prevention and, if necessary, the treatment of such conditions; it is a topic which also has medico-legal implications. It was therefore most useful to have a group of papers which dealt with the consequences of acute and chronic exposure of humans to ionizing radiation.

The subject of the epidemiology of radiation workers generated a certain amount of discussion during this symposium. I am sure that I do not need to remind this distinguished audience of Milton's words 'argument and disputation amongst persons of good will are but knowledge in the making' (actually he wrote 'men of good will' but we have progressed since 1644). It is therefore possible that we have made a little knowledge during the past week.

Let me attempt to summarize the situation as I see it:

- (i) Few of us believe that occupational exposure to ionizing radiation at currently usual levels constitutes an unacceptable risk — and I speak as a radiation worker;
- (ii) Equally, few of us at present can adequately document this belief;
- (iii) We are being required by the public and by regulatory bodies to provide this kind of documentation.

It therefore follows that many of us are going to be involved in epidemiology over the next several decades.

Much of the discussion during the sessions has centred on the difficulty of performing such studies as part of a sufficiently rigid scientific procedure. I suggest that we should attempt to determine by follow-up whether there is any evidence of a difference in the causes of and age at death of workers exposed to different levels of radiation. This is the question that has been put to the United Kingdom Registry and I think it is a good one.

Somewhere between uncritical speculation and insistence upon impossible mathematical certainty we must look for a degree of certitude where interpretations and propositions can be established beyond reasonable, although not necessarily all possible, doubt. This degree of certitude depends upon the disciplined use of evidence.

It is to the gathering and interpretation of this evidence that we must now address ourselves. Recognizing that many countries will probably embark on programmes of this type, we must try to avoid a situation in which it becomes impossible eventually to compare the results of these studies. I hope that it will be possible for the international community to convene a meeting at which criteria for the study of the health of radiation workers can be determined. If such criteria can be adopted as guidelines by the nuclear countries of the world, we may well have simplified the task of those who follow in our footsteps.

A.S. McLEAN (*Chairman*): That concludes the chairmen's reports on the sessions. This is the first time I have experienced this summarizing procedure at symposia and I think it is a most useful exercise which I would commend to other Divisions of the Agency. Now, before opening the discussion to the floor, I should like to call on Mr. Volodin from the World Health Organization who, I believe, wishes to say a few words to us.

V.M. VOLODIN: In pursuance of World Health Assembly resolutions on radiation protection and the biological effects of radiation, the World Health Organization has for many years been concerned with the problems of assessing and preventing harmful effects of radiation on human beings. In this connection I should like to draw your attention to some recommendations of WHO's Scientific Group on the Long-term Effects of Radium and Thorium in Man which was

established in September 1977, as some of these recommendations would seem relevant to questions raised at this symposium. I quote:

The Scientific Group recommends that in relation to world public health, it is most important to continue and complete the epidemiological investigations in man — and the biophysical and medical studies associated with them — which are now in progress in a number of countries to evaluate the effects of internally incorporated alpha-emitters such as thorotrast, $^{224}\text{Ra}(\text{ThX})$, ^{226}Ra , $^{228}\text{Ra}(\text{MsThI})$, radon (^{222}Rn), thoron (^{220}Rn), ^{232}Th , plutonium and higher actinides, with special emphasis to examination of combined effects due to the radiations in the presence of other agents, such as alcohol and as far as possible, smoking, together with industrial dusts and some other apparently inert materials.

Unfortunately, we cannot at present provide the essential financial support for these studies as there are many other very important problems calling for WHO's attention. However, we believe that national investigations into problems and difficulties should be supported in every possible way either through bilateral collaboration, or by assistance and encouragement from national intergovernmental and international organizations concerned with the development of the peaceful applications of nuclear energy.

A.S. McLEAN (*Chairman*): Thank you, Mr. Volodin. Now, are there any questions or comments from the floor?

J. BERGSMAN: A new development in nuclear medicine is the Amy Scan computer tomography by absorbing X-rays; for example, an abdominal examination takes only 18 seconds and a very clear picture is obtained. A few months ago, at the 15th annual meeting of the Society of Nuclear Medicine in Groningen, Netherlands, a whole day was spent discussing tomography. However, no clear indication was given as to the radiation dose from tomography and I wonder if anyone here could say what the absorbed dose to the patient is from tomography of the head, thorax and abdomen?

A.S. McLEAN (*Chairman*): I think there was a paper from a United States laboratory in Health Physics in January on the doses from scanners. We have also made some measurements at our laboratory and I should be pleased to supply you with the data.

A.B. DORY: I should like to appeal to all concerned in this field to focus attention on the investigation of the delayed biological effects of low-level alpha emitters which constitute quite a big problem in connection with uranium mining, particularly in combination with various other agents such as silica dust, diesel fumes, etc. I should also like to re-emphasize the need for caution in drawing conclusions from the results of animal studies. It is essential to put these into proper perspective. The aim of our group, as I see it, is to try and foresee what

kind of effects might result from activities in the nuclear field, and to protect the general population and people whose occupation exposes them to certain radiation risks. Therefore I think it is important that scientists should start talking to the people whom it is their task to protect in terms that they can understand because, if you leave the explanation of the hazards of radiation to pseudoscientists, you will get the sort of situation, which is already very evident in North America and now spreading to Europe as well, where people simply have no idea what the real hazards are.

A.S. McLEAN (*Chairman*): Yes, I think we have a lot to learn about communication.

Now, if there are no further comments I should like to conclude by offering you my impressions of the week's events and the general scene.

It has long been evident that many of the most difficult and central problems in radiological protection derive from uncertainties about the biological effects of ionizing radiations delivered slowly and for long periods of time. One might have hoped that the massive amount of research and investigation that has marked the past thirty-five years or so would have led us to the point where hypothesis would have largely given way to fact, so that areas of important uncertainty and therefore of potential conflict, would by now be minimal. However, in spite of the best endeavours of scientists in laboratories, national research institutes and international organizations, notably UNSCEAR, this happy stage often seems to be as far away as ever.

It is scarcely surprising, therefore, that one of the results of what is often called the nuclear debate has been to encourage those who, for one reason or another, would exaggerate or belittle the hazards of radiation, thus sharpening the controversy.

This meeting has brought together people with a remarkably wide range of interests including the practicalities of radiological protection, epidemiology and theoretical and experimental radiobiology; I believe that it is for the radiobiologists in particular that this symposium has been arranged. The essential nature of their role has been apparent this week in that many of the continuing problems clearly lie in the manner in which radiobiological considerations are reflected in protection policies and regimes. This is certainly so in the case of the system of dose limitation recommended recently by ICRP in relation both to the assessment of detriment (in order to make it possible to compare detriment with benefit) and to the choice of numerical values for dose limits. I hope that this symposium will prove to have had some influence on the selection of subjects for further research.

Among the topics that appear to me to be of particular importance are: the shape of the dose-response curves for low doses of various qualities of radiation and particularly for low LET radiation; the clinical significance of radiation-induced chromosome lesions; combined effects; inhomogeneous irradiation; and further elucidation of the genetic effects of radiation.

Turning now to epidemiological aspects, which have been featured this week, it is clear that yet more information about the late effects of acute irradiation will be forthcoming from studies on the Japanese survivors. As far as low-dose protracted irradiation is concerned, however, the field is wide open as we heard in the evening discussion. If, as seems probable, machinery is to be set up on a wide scale for the collection and analysis of data relating to occupational exposure, particularly in nuclear establishments, then the first requirement must surely be a clear appreciation of the aims of such schemes in the context of what can reasonably be achieved. I would like to make a plea for simplicity. Simplicity would, in my view, limit such schemes to the recording of essential data and would rule out, for example, large-scale cytogenetic investigations and elaborate systems of dosimetry. In any event, what is done in relation to radiation should be consistent with principles adopted in relation to the wide range of occupational carcinogens.

Similarly, epidemiologists will do much to gain the support of their scientific colleagues as well as intelligent laymen if they will address themselves to the centrally important matter of presenting the results of their analyses simply and consistently.

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The following conversion table is provided for the convenience of readers and to encourage the use of SI units.

FACTORS FOR CONVERTING SOME OF THE MORE COMMON UNITS TO INTERNATIONAL SYSTEM OF UNITS (SI) EQUIVALENTS

NOTES:

- (1) SI base units are the metre (m), kilogram (kg), second (s), ampere (A), kelvin (K), candela (cd) and mole (mol).
- (2) ► indicates SI derived units and those accepted for use with SI;
 ► indicates additional units accepted for use with SI for a limited time.
 [For further information see *The International System of Units (SI)*, 1977 ed., published in English by HMSO, London, and National Bureau of Standards, Washington, DC, and International Standards ISO-1000 and the several parts of ISO-31 published by ISO, Geneva.]
- (3) The correct abbreviation for the unit in column 1 is given in column 2.
- (4) * indicates conversion factors given exactly; other factors are given rounded, mostly to 4 significant figures.
 ≡ indicates a definition of an SI derived unit: [] in column 3+4 enclose factors given for the sake of completeness.

Column 1 Multiply data given in:	Column 2	Column 3 by:	Column 4 to obtain data in:
Radiation units			
► becquerel disintegrations per second (= dis/s)	1 Bq	(has dimensions of s^{-1})	
► curie	1 s^{-1}	$\equiv 1.00 \times 10^0$	Bq *
► roentgen	1 Ci	$\equiv 3.70 \times 10^{10}$	Bq *
► gray	1 R	$[= 2.58 \times 10^{-4}$	C/kg] *
► rad	1 Gy	$[= 1.00 \times 10^0$	J/kg] *
sievert (radiation protection only)	1 rad	$= 1.00 \times 10^{-2}$	Gy *
rem (radiation protection only)	1 Sv	$[= 1.00 \times 10^0$	J/kg] *
	1 rem	$[= 1.00 \times 10^{-2}$	J/kg] *
Mass			
► unified atomic mass unit ($\frac{1}{12}$ of the mass of ^{12}C)	1 u	$[= 1.660\,57 \times 10^{-27}$	kg, approx.]
► tonne (= metric ton)	1 t	$[= 1.00 \times 10^3$	kg] *
pound mass (avoirdupois)	1 lbm	$= 4.536 \times 10^{-1}$	kg
ounce mass (avoirdupois)	1 ozm	$= 2.835 \times 10^1$	g
ton (long) (= 2240 lbm)	1 ton	$= 1.016 \times 10^3$	kg
ton (short) (= 2000 lbm)	1 short ton	$= 9.072 \times 10^2$	kg
Length			
statute mile	1 mile	$= 1.609 \times 10^0$	km
nautical mile (international)	1 n mile	$= 1.852 \times 10^0$	km *
yard	1 yd	$= 9.144 \times 10^{-1}$	m *
foot	1 ft	$= 3.048 \times 10^{-1}$	m *
inch	1 in	$= 2.54 \times 10^1$	mm *
mil ($= 10^{-3}$ in)	1 mil	$= 2.54 \times 10^{-2}$	mm *
Area			
► hectare	1 ha	$[= 1.00 \times 10^4$	m^2] *
► barn (effective cross-section, nuclear physics)	1 b	$[= 1.00 \times 10^{-28}$	m^2] *
square mile, (statute mile) ²	1 mile ²	$= 2.590 \times 10^0$	km^2
acre	1 acre	$= 4.047 \times 10^3$	m^2
square yard	1 yd ²	$= 8.361 \times 10^{-1}$	m^2
square foot	1 ft ²	$= 9.290 \times 10^{-2}$	m^2
square inch	1 in ²	$= 6.452 \times 10^2$	mm^2
Volume			
► litre	1 l or 1 ltr	$[= 1.00 \times 10^{-3}$	m^3] *
cubic yard	1 yd ³	$= 7.646 \times 10^{-1}$	m^3
cubic foot	1 ft ³	$= 2.832 \times 10^{-2}$	m^3
cubic inch	1 in ³	$= 1.639 \times 10^4$	mm^3
gallon (imperial)	1 gal (UK)	$= 4.546 \times 10^{-3}$	m^3
gallon (US liquid)	1 gal (US)	$= 3.785 \times 10^{-3}$	m^3
Velocity, acceleration			
foot per second (= fps)	1 ft/s	$= 3.048 \times 10^{-1}$	m/s *
foot per minute	1 ft/min	$= 5.08 \times 10^{-3}$	m/s *
mile per hour (= mph)	1 mile/h	$= \begin{cases} 4.470 \times 10^{-1} \\ 1.609 \times 10^0 \end{cases}$	$\begin{matrix} \text{m/s} \\ \text{km/h} \end{matrix}$
► knot (international)	1 knot	$= 1.852 \times 10^0$	km/h *
free fall, standard, g		$= 9.807 \times 10^0$	m/s^2
foot per second squared	1 ft/s ²	$= 3.048 \times 10^{-1}$	m/s^2 *

This table has been prepared by E. R. A. Beck for use by the Division of Publications of the IAEA. While every effort has been made to ensure accuracy, the Agency cannot be held responsible for errors arising from the use of this table.

Column 1 Multiply data given in:	Column 2	Column 3 by:	Column 4 to obtain data in:
Density, volumetric rate			
pound mass per cubic inch	1 lbm/in ³	= 2.768 × 10 ⁴	kg/m ³
pound mass per cubic foot	1 lbm/ft ³	= 1.602 × 10 ¹	kg/m ³
cubic feet per second	1 ft ³ /s	= 2.832 × 10 ⁻²	m ³ /s
cubic feet per minute	1 ft ³ /min	= 4.719 × 10 ⁻⁴	m ³ /s
Force			
newton	1 N	[≡ 1.00 × 10 ⁰	m · kg · s ⁻²] *
dyne	1 dyn	= 1.00 × 10 ⁻⁵	N *
kilogram force (= kilopond (kp))	1 kgf	= 9.807 × 10 ⁰	N
poundal	1 pdl	= 1.383 × 10 ⁻¹	N
pound force (avoirdupois)	1 lbf	= 4.448 × 10 ⁰	N
ounce force (avoirdupois)	1 ozf	= 2.780 × 10 ⁻¹	N
Pressure, stress			
pascal	1 Pa	[≡ 1.00 × 10 ⁰	N/m ²] *
atmosphere ^a , standard	1 atm	= 1.013 25 × 10 ⁵	Pa *
bar	1 bar	= 1.00 × 10 ⁵	Pa *
centimetres of mercury (0°C)	1 cmHg	= 1.333 × 10 ³	Pa
dyne per square centimetre	1 dyn/cm ²	= 1.00 × 10 ⁻¹	Pa *
feet of water (4°C)	1 ftH ₂ O	= 2.989 × 10 ³	Pa
inches of mercury (0°C)	1 inHg	= 3.386 × 10 ³	Pa
inches of water (4°C)	1 inH ₂ O	= 2.491 × 10 ²	Pa
kilogram force per square centimetre	1 kgf/cm ²	= 9.807 × 10 ⁴	Pa
pound force per square foot	1 lbf/ft ²	= 4.788 × 10 ¹	Pa
pound force per square inch (= psi) ^b	1 lbf/in ²	= 6.895 × 10 ³	Pa
torr (0°C) (= mmHg)	1 torr	= 1.333 × 10 ²	Pa
Energy, work, quantity of heat			
joule (≡ W · s)	1 J	[≡ 1.00 × 10 ⁰	N · m] *
electronvolt	1 eV	[= 1.602 19 × 10 ⁻¹⁹	J, approx.]
British thermal unit (International Table)	1 Btu	= 1.055 × 10 ³	J
calorie (thermochemical)	1 cal	= 4.184 × 10 ⁰	J *
calorie (International Table)	1 cal _{IT}	= 4.187 × 10 ⁰	J
erg	1 erg	= 1.00 × 10 ⁻⁷	J *
foot-pound force	1 ft · lbf	= 1.356 × 10 ⁰	J
kilowatt-hour	1 kW · h	= 3.60 × 10 ⁶	J *
kiloton explosive yield (PNE) (≡ 10 ¹² g-cal)	1 kt yield	≈ 4.2 × 10 ¹²	J
Power, radiant flux			
watt	1 W	[≡ 1.00 × 10 ⁰	J/s] *
British thermal unit (International Table) per second	1 Btu/s	= 1.055 × 10 ³	W
calorie (International Table) per second	1 cal _{IT} /s	= 4.187 × 10 ⁰	W
foot-pound force/second	1 ft · lbf/s	= 1.356 × 10 ⁰	W
horsepower (electric)	1 hp	= 7.46 × 10 ²	W *
horsepower (metric) (= ps)	1 ps	= 7.355 × 10 ²	W
horsepower (550 ft · lbf/s)	1 hp	= 7.457 × 10 ²	W
Temperature			
temperature in degrees Celsius, t where T is the thermodynamic temperature in kelvin and T ₀ is defined as 273.15 K	t = T - T ₀		
degree Fahrenheit	t _{°F} - 32		
degree Rankine	T _{°R}		
degrees of temperature difference ^c	ΔT _{°R} (= Δt _{°F})		
$\left. \begin{array}{l} t_{°F} - 32 \\ T_{°R} \\ \Delta T_{°R} (= \Delta t_{°F}) \end{array} \right\} \times \left(\frac{5}{9} \right) \text{ gives } \left\{ \begin{array}{l} t \text{ (in degrees Celsius) } * \\ T \text{ (in kelvin) } * \\ \Delta T (= \Delta t) \end{array} \right.$			
Thermal conductivity ^c			
1 Btu · in/(ft ² · s · °F) (International Table Btu)	= 5.192 × 10 ²		W · m ⁻¹ · K ⁻¹
1 Btu/(ft · s · °F) (International Table Btu)	= 6.231 × 10 ³		W · m ⁻¹ · K ⁻¹
1 cal _{IT} /(cm · s · °C)	= 4.187 × 10 ²		W · m ⁻¹ · K ⁻¹

^a atm abs, ata: atmospheres absolute;
atm (g), atü: atmospheres gauge.

^b lbf/in² (g) (= psig): gauge pressure;
lbf/in² abs (= psia): absolute pressure.

^c The abbreviation for temperature difference, deg (= degK = degC), is no longer acceptable as an SI unit.

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